Section 5:
Crop Biology and Disease Epidemiology

OPTIMIZING GRAPE ROOTSTOCK PRODUCTION AND EXPORT OF INHIBITORS OF *XYLELLA FASTIDIOSA* POLYGALACTURONASE ACTIVITY

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ABSTRACT
In response to a recommendation by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Research Scientific Advisory Panel to express plant genes for particularly effective inhibitors of *Xylella fastidiosa* (*Xf*) polygalacturonase (PG) in transgenic grape, optimal plant polygalacturonase inhibiting proteins (PGIPs) are being selected and expressed in grape rootstocks to enhance grapevine Pierce's disease (PD) resistance. This project includes integrated approaches aimed at the eventual deployment of that strategy in commercial lines. To ease the path to commercialization, PIPRA investigators are examining the relevant Intellectual Property and regulatory issues associated with the use of selected PGIPs in transgenic grape rootstocks in combination with elite scion lines. The PGIPs that most effectively inhibit *Xf*PG are predicted to be the best candidates for providing significant PD resistance. Recombinant *Xf*PG is being developed to screen diverse PGIPs selected from a wide variety of plant sources for their ability to effectively inhibit the *Xf*PG enzyme. We are cloning the selected PGIPs so they can be expressed in plants for the tests of their efficacy in inhibiting *Xf*PG. Grape rootstock lines will be transformed with the most effective PGIPs and signal and target sequences will be used as needed to maximize PGIP expression in the rootstock and its export to the non-transgenic scions. At the conclusion of the project, the capacity of non-transgenic scions to resist PD and produce high quality grapes when grafted on transgenic rootstocks will be tested.

LAYPERSON SUMMARY
Plant proteins have been selected to inhibit a key enzyme called polygalacturonase (PG) that *Xylella fastidiosa (Xf)* uses to spread from the point of inoculation throughout the grapevine and cause Pierce's disease (PD). Proteins called PG-inhibiting proteins (PGIPs) are produced by many plants. PGIPs are selective in their ability to inhibit the PG enzymes of plant pathogens. We know that the pear fruit PGIP can inhibit *Xf*PG and that expression of the pear PGIP in transgenic grapevines slow PD development. We also know that pear PGIP produced in a rootstock can move into scions by crossing the graft union in the water-conducting tissues. The PGIPs from different plants are being tested for their ability to inhibit *Xf*PG and structural modeling is being used to characterize what parts of the PGIP are important for inhibition. The best inhibiting PGIPs will be expressed in grape and their ability to reduce PD development in grafted scions will be determined. Regulations regarding the release and use of transgenic rootstocks and intellectual property considerations associated with this approach are being addressed to maximize the commercial potential of this PD management strategy, an approach that has been advocated by the CDFA PD/GWSS Advisory Panel.

INTRODUCTION
Xylella fastidiosa (Xf), the causative agent of Pierce's disease (PD) in grapevines, has been detected in infected portions of vines. Several lines of evidence support the hypothesis that *Xf* uses cell wall-degrading enzymes to digest the polysaccharides of plant pit membranes separating the elements of the water-conducting vessel system, the xylem, of the vines (Thorne et al., 2006). *Xf* cell wall degrading enzymes break down these primary cell wall barriers between cells in the xylem, facilitating the systemic spread of the pathogen. The genome of *Xf* contains genes predicted to encode a polygalacturonase (PG) and several β-1,4-endo-glucanases (EGase), cell wall degrading enzymes that are known to digest cell wall pectin and xyloglucan polymers, respectively. To demonstrate that these wall degrading proteins facilitate *Xf* systemic movement and PD development, Roper et al. (2007) developed a PG-deficient strain of *Xf* and showed that the mutant bacterial strain was unable to cause PD symptoms; thus, the *Xf*PG is a virulence factor of the bacteria that contributes to the development and spread of PD. Labavitch et al. (2006) reported that introduction of PG and EGase enzymes into explanted stems of uninfected grapevines caused breakage of the pit membranes and demonstrated that substrates for these enzymes, pectins and xyloglucans, are present in grapevine pit membranes (Labavitch, 2007).

PG-inhibiting proteins (PGIPs) produced by plants limit damage caused by fungal pathogens (*B. cinerea*, or gray mold) as well as by insects (*Lygus hesperus*, the western tarnished plant bug) (Powell et al., 2000; Shackel et al., 2005) because PGIPs

are selective inhibitors of the PGs produced by fungal pathogens and insects (Cervone et al., 1990). Agüero et al. (2005) demonstrated that by introducing a pear fruit PGIP gene (Stotz et al., 1993; Powell et al., 2000) into transformed grapevines, the susceptibility to both fungal (*B. cinerea)* and bacterial (*Xf)* pathogens decreased. This result implied that the pear PGIP provided protection against PD by inhibiting the *Xf* PG, reducing its efficiency as a virulence factor. Using *in vitro* assays with *Xf*PG expressed in *E. coli*, Roper (2006) demonstrated that the recombinantly expressed *Xf*PG can be inhibited by the pear PGIP (Labavitch, 2006). In a key preliminary observation for the PD control approach investigated in this project, Agüero et al. (2005) demonstrated that transgenic pear PGIP protein could be transported across a graft junction of genetically engineered grapevines into the aerial portions of wild-type scions.

The overall goal of the project is to develop transgenic grape rootstock lines that optimally express PGIPs that most effectively reduce the virulence of *Xf*.. The project is designed to identify specific PGIPs that optimally inhibit the virulence factor, *Xf*PG, and to express efficiently the optimal PGIPs in grape rootstocks to provide PD protection in scions. The optimization of the expression of PGIPs includes the use of transformation components with defined intellectual property (IP) and regulatory characteristics, as well as expression regulating sequences that result in the maximal production of the PGIPs in rootstocks and efficient transport of the proteins through the graft junctions to the aerial portions of the vines so that *Xf*PG produced by the pathogen in scions is inhibited.

OBJECTIVES

1. Define a path to commercialization of a PD control strategy using PGIPs, focusing on IP and regulatory issues associated with the use of PGIPs in grape rootstocks.
2. Identify plant PGIPs that maximally inhibit *Xf*PG
3. Assemble transcription regulatory elements, *Xf*-inducible promoters, and signal sequences that maximize PGIP expression in and transport from roots.
4. Create PGIP-expressing rootstocks and evaluate their PD resistance.

RESULTS AND DISCUSSION

Objective 1: Regulatory issues associated with commercialization of transgenic rootstocks

A visit by PIPRA staff to federal agencies regulating the environmental release into the environment of genetically modified (GM) plants gave us insights on the regulatory issues related to *PGIP* expressing grape rootstocks. From a USDA/APHIS perspective, it will be recommended that field trials start with GM rootstocks and wild type scions will be grafted on later for deregulation of future commercial products. From an EPA standpoint, one potential issue for this project to address will be gene-flow from transgenic pollen. For regulatory approval only, it will be necessary to allow rootstocks to flower in commercial settings even though under normal practices, the rootstocks will not be allowed to flower. The EPA will have the final word on defining if grapes harvested from non-GM scions grafted on the *PGIP*-expressing rootstocks will be considered transgenic. This will determine if the FDA needs to be consulted before commercialization.

Objective 2: Propagation and grafting of existing grape lines expressing and exporting pear PGIP

Agüero et al. (2005) described the use of transgenic grapevine cultivars 'Thompson Seedless' and 'Chardonnay' expressing the 'Bartlett' pear fruit PGIP (*PcBPGIP*). These plants have been maintained in our greenhouse facilities with the intent to use them in grafting and *Xf* inoculation experiments. Vegetative propagation efforts to increase the total number of plants for these experiments yielded 66% efficiency last winter. PCR analysis has been used to verify the transgene identity in both grape cultivars containing either the *PcBPGIP* transgene or the empty vector.

Further work related to this objective has been delayed substantially due to quarantine measures implemented in response to a Panicle Rice Mite infestation in the greenhouse facilities. For more information regarding these matters, please refer to the August 2009 progress report for CDFA contract number 08-0171. The CDFA and UC Davis issued directives for treating the affected grape vines including drastic pruning and isolation, resulting in rootstocks with only one or two viable buds remaining prior to intensive chemical disinfestations treatments. Some of the

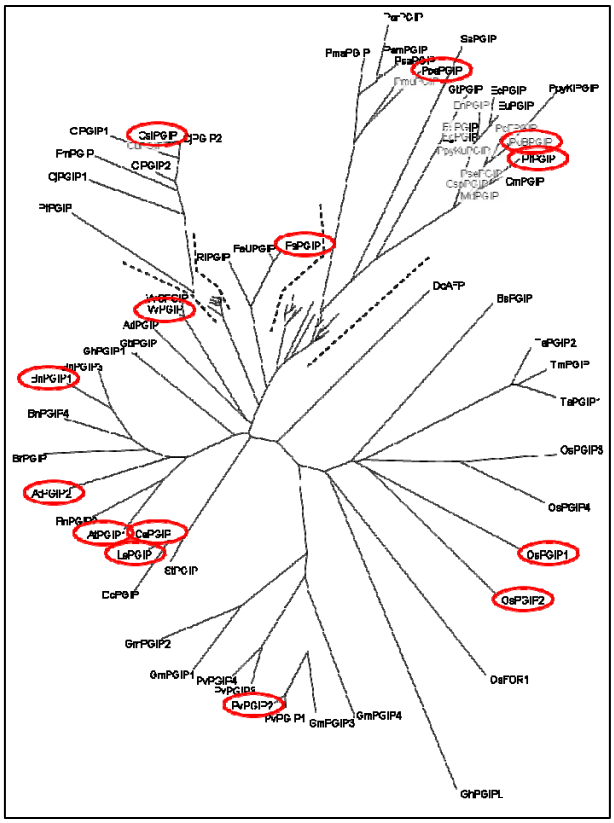

Figure 1. Unrooted phylogenetic tree of PGIP proteins. The 14 candidate PGIPs for *Xf*PG inhibition are circled in red. The protein names and organisms are given in Labavitch, 2008.

grape lines did not survive the mandated action and all remaining lines have shown slower than normal regeneration. Due to the considerable amount of time and effort to follow these directives and regenerate the plants, the grafting experiments to be conducted in years 1 and 2 have been delayed until the affected grapevines can be moved back to the appropriate facilities. As of October 2009, the grapevines remain in isolation.

Objective 2: Selection of PGIPs

The previously reported phylogenetic tree of PGIP sequences (Labavitch 2008) has been expanded upon to include a total of 68 PGIP-like amino acid sequences. These PGIPs represent a diverse array of plant families and expression patterns. The PG inhibition activities of some of them are known. The full-length protein sequences from GenBank were aligned using ClustalX 2.0.9. An unrooted, neighbor-joining tree (**Figure 1**) was constructed in ClustalX and visualized with TreeView 1.6. PGIPs are typically characterized by 10 leucine rich repeats (LRR) in the region thought to influence inhibition of PGs. The PGIP sequence diversity in this phylogenetic tree mirrors the diversity among plant families, crediting the use of PGIP sequence data in plant family classification studies.

Fourteen candidate PGIPs (**Figure 1, Table 1**) were selected from the phylogeny, representing the major clades of the tree and the inherent sequence variation dividing them. The candidates were also chosen by their predicted total protein charge at a given pH. The predicted charges were calculated for all 68 PGIP sequences but the lower total charges predicted for the candidates should prevent interference or repulsion between each PGIP and the highly charged *Xf*PG. The large positive charge on AtPGIP2 and the minimal charge on OsPGIP2 will be particularly informative as we correlate *Xf*PG inhibition with total PGIP charge.

Table 1. Predicted total protein charge analysis for the 14 candidate PGIPs and *Xf*PG in different pH environments.

Common name	Organism	Protein	Charge of Protein (at certain pH)					
			3.5	*4.0*	***4.5***	*5.0*	*5.5*	*6.0*
Thale cress	*Arabidopsis thaliana* (Col.)	AtPGIP1	27.5	20.9	**14.2**	10.0	7.4	5.2
Thale cress	*Arabidopsis thaliana* (Col.)	AtPGIP2	35.4	28.5	**21.6**	17.0	14.2	11.8
Rapeseed	*Brassica napus* cv. DH12075	BnPGIP1	30.5	22.2	**14.2**	9.4	6.8	4.8
Pepper	*Capsicum annum* cv. arka abhir	CaPGIP	20.7	15.2	**9.5**	5.9	3.8	2.2
Sweet orange	*Citrus sinensis* cv. Hamlin	CsiPGIP	28.0	21.7	**15.2**	11.1	8.7	6.7
Strawberry	*Fragaria x ananassa*	FaPGIP	25.4	18.7	**12.1**	8.0	5.6	3.7
Rice	*Oryza sativa* cv. Roma	OsPGIP1	18.4	12.9	**7.6**	4.3	2.2	0.2
Rice	*Oryza sativa* cv. Roma	OsPGIP2	17.5	9.3	**1.6**	-3.1	-6.1	-8.8
Common bean	*Phaseolus vulgaris* cv. Pinto	PvPGIP2	22.7	17.6	**12.9**	10.2	8.5	7.1
Peach	*Prunus persica*	PpePGIP	28.7	21.9	**14.9**	10.3	7.5	5.3
Chinese Firethorn	*Pyracantha fortuneana*	PfPGIP	16.9	11.7	**6.6**	3.4	1.4	-0.3
Bartlett pear	*Pyrus communis* cv. Bartlett	PcBPGIP	23.1	16.1	**9.3**	5.0	2.6	0.7
Tomato	*Solanum lycopersicum* cv. VFNT Cherry	LePGIP	29.8	23.4	**17.0**	12.8	10.1	7.7
Grape	*Vitis vinifera* cv. Pinotage	VvPGIP	30.5	24.0	**17.7**	13.6	11.1	8.7
		*Xf*PG	41.0	31.3	**22.2**	16.4	11.9	6.8

Objective 2: Express PGIPs and test for optimal inhibition of XfPG

The 14 candidate PGIPs will be tested for their ability to inhibit *Xf*PG. The previously reported plant transformation strategy (Labavitch 2008) is being used to generate plant transformation vectors containing a PGIP sequence under control of the CaMV 35S constitutive promoter and linked to a C-terminal poly-His tag for protein purification. These plant proteins are highly glycosylated (**Figure 3B**) and therefore require expression in a plant-based system. Arabidopsis lines transformed to express each PGIP will be used to obtain the proteins necessary for *in vitro* radial diffusion assays, testing the inhibitory capacity of each PGIP. Cloning each of the candidate PGIPs from its source species into the proper plant transformation vector is in progress (**Table 2**). The stably expressing Arabidopsis lines will provide PGIPs for inhibition assays against the PD causing *Xf*PG, as well as against PGs from other pathogens and pests linked to many plant diseases and resulting crop losses.

Table 2. Research progress for cloning the 14 candidate PGIPs. "X" = completed checkpoint, "O" = work in progress, "-"= checkpoint to be completed.

Protein	Cloning Progress Checkpoints				
	Plant tissue acquired	PGIP cDNA isolated	Transformed into *E. coli*	Transformed into *A. tumefaciens*	PGIP ready for plant transformation
AtPGIP1	X	X	X	O	-
AtPGIP2	X	X	X	O	-
BnPGIP1	O	-	-	-	-
CaPGIP	X	O	-	-	-
CsiPGIP	X	O	-	-	-
FaPGIP	X	X	-	-	-
OsPGIP1	X	O	-	-	-
OsPGIP2	X	O	-	-	-
PvPGIP2	X	O	-	-	-
PpePGIP	O	-	-	-	-
PfPGIP	X	O	-	-	-
PcBPGIP	X	X	X	X	O
LePGIP	X	X	X	X	O
VvPGIP	O	-	-	-	-

The *in vitro* assays require optimal expression and activity of *Xf*PG, a topic covered below. We are developing another assay to test each candidate PGIP's ability to inhibit *Xf*PG *in planta*. This assay will provide an environment more similar to the potential PG-PGIP interaction taking place in the plant apoplastic space. Separate plant transformation vectors carrying a candidate PGIP and the *Xf*PG coding sequence will be used to transiently co-express both proteins in tobacco leaves by *Agrobacterium tumefaciens* pressure infiltration. An analogous assay was used to test the effectiveness of a grape PGIP (VvPGIP) in inhibiting a PG from *B. cinerea* (Joubert et al., 2007). Both the PG and PGIP were transiently expressed in tobacco leaves by co-infiltration of *A. tumefaciens* clones carrying the genes of interest. Expression of the PG alone resulted in PG-dependent lesions which were visible and could be measured. Co-infiltration of PG and PGIP-expressing clones resulted in inhibition of PG-mediated lesion development (**Figure 2**). *Xf* is known to cause local lesions in tobacco leaves after infection. Two plant transformation constructs have been developed containing *Xf*PG for this experiment: one with the native coding sequence and one with an apoplastic targeting sequence attached upstream of the coding region to ensure *Xf*PG secretion by the plant cells. We expect to see results similar to those from the earlier work: local lesions induced by the expression of *Xf*PG will be lessened when the *Xf*PG is co-expressed with an inhibiting PGIP. This will support rapid comparisons of the effectiveness of each PGIP in inhibiting *Xf*PG *in planta*.

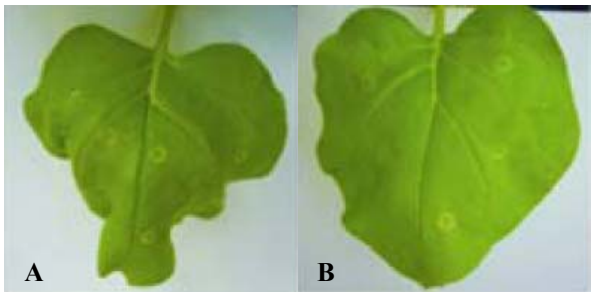

Figure 2. Co-expression of *Bcpg2* with either (A) empty vector control or (B) *Vvpgip* in *N. benthamiana* leaves at 24 h postinfiltration. Leaves were infiltrated with the two *A. tumefaciens* strains in a 1:1 ratio. Figure from Joubert et al., 2007.

Objective 2: XfPG cloning and expression

The *Xf*PG gene was subcloned from the pET29b vector into pMT/BiP/V5-HisA, a vector compatible with the Drosophila protein expression system used by R. Booth. The construct was confirmed by performing digestions with EcoRI, XhoI, EcoRV, and sequencing by the UC Davis DNA Sequencing facility. Transfections were performed with the confirmed *Xf*PG construct or an expression vector containing a GFP marker as the positive control; non-transfected cells were tested as the negative control. Cellular components (pellet) and supernatant (SN) from the transfected lines were collected. Protein expression was validated by SDS-PAGE and Western Blot analyses (**Figure 3**). The *Xf*PG protein has an apparent molecular weight of 70 kD, slightly greater than expected, possibly due to the effects of glycosylation. Crude extracts will be assayed for PG activity while further steps to purify the protein using the attached His-tag will provide *Xf*PG for future *in vitro* PGIP inhibition assays.

Figure 3. Lanes 1-4 are protein collected from the SN of the transfection reaction. Lanes 5-8 are protein collected from the pellet. Lanes 1, 5: negative control; lanes 2, 3, 6, 7: *Xf*PG transfection; lanes 4, 8: positive control of GFP marker-expressing cells.

Objective 2: Model PGIP and XfPG interactions to aid in optimal PGIP prediction for PD defense
The interaction between PG and PGIP proteins influences whether the plant PGIP is able to successfully inhibit the pathogen virulence factor, *XfPG*. The crystal structure of PvPGIP2 (Di Matteo et al., 2003) has facilitated structural inquiries into what regions of the PGIP are responsible for PG inhibition. One study found that a single amino acid, Q224, is responsible for *Fusarium moniliforme* PG (*Fm*PG) inhibition by PvPGIP2 by comparison to PvPGIP1, which is unable to inhibit *Fm*PG (Leckie et al., 1999). While sequence variation can account for some of the specificity, the ability of PvPGIP2 to have competitive, non-competitive, and mixed modes of inhibition for *Fm*PG, *A. niger* PGII, and *B. cinerea* PG1, respectively, suggests that additional recognition and specificity sequences or motifs occur (Federici et al., 2001; King et al., 2002; Sicilia et al., 2005). PGIPs are heavily glycosylated proteins with 7 potential N-linked glycosylation sites on PcBPGIP (Lim et al., 2009) thereby adding 14.5 kD to the molecular weight (Powell et al., 2000). It has been hypothesized that differing glycosylation patterns also affect PGIP specificity.

Homology modeling efforts by D. King created *in silico* interactions between the predicted structures of *Xf*PG and each of the 14 candidate PGIPs to visualize the possible interactions and predict the likelihood of a successful inhibition. Each structural model was created by threading the PGIP amino acid sequence onto the PvPGIP2 crystal structure. Models were then optimized with molecular mechanics, MM3, using the Swiss PDB Viewer DeepView 3.7 and the modeling suite BioMedCAChe 6.1 (**Figure 4A**). Glycosylated versions of the models were created by attaching three $Man_3XylGlcNAc_2$ and four $Man_3XylGlcNAc_2Fuc$ groups to the appropriate sites (NxS/T) on the optimized protein structures as previously determined for PcBPGIP (**Figure 4B**; Lim et al., 2009). The putative *Xf*PG model was visualized and optimized with the same techniques used for each PGIP.

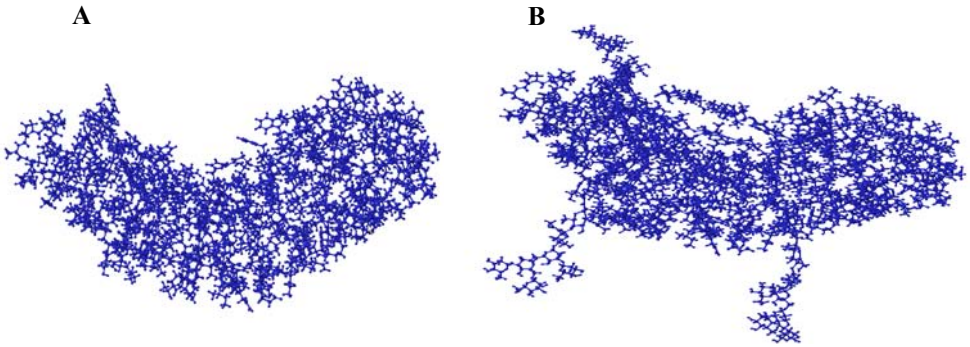

Figure 4. Homology models of (A) the PcBPGIP protein and (B) the protein with N-linked glycosylations. The concave face of the PGIP is thought to interact with PGs.

The inhibitory capability of each PGIP was determined through a series of dynamic reaction simulations where the effectiveness of the potential inhibition was measured by the ability of amino acids or glycosylations on the PGIPs to block key amino acids on the surface of the *Xf*PG that are responsible for cleaving the modeled polygalacturonan (PGA) substrate. The *Xf*PG model was put through a series of simulations with the PGA in its active cleft and keeping various groups of amino acid residues on the outer β sheet locked in place until immobilizing a particular group of residues inhibited the enzyme's *in silico* cleavage of the substrate (**Figure 5A**). It was determined that two clusters of amino acids, 63-74 & 223-226, control the ability of *Xf*PG to cleave its substrate. Dynamic reaction simulations were carried out with the PG, PGIP, and PGA substrate to determine if the PGA was cleaved and therefore, to what extent the PGIP inhibited the PG (**Figure 5B**). The dynamic reaction simulations were supplemented by preliminary surface chemistry mapping in BioMedCAChe to determine

if compatible acid/base regions were present on the LRR face of the PGIP and the previously demonstrated controlling region of *Xf*PG. Both techniques identified PcBPGIP, CsiPGIP, and OsPGIP1 as the potentially most effective inhibitors of *Xf*PG.

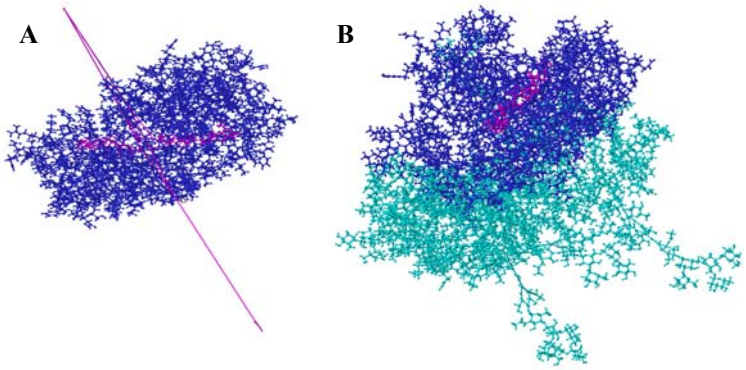

Figure 5. *Xf*PG homology models. (A) *Xf*PG (blue) with PGA (pink) being cleaved in its active site. (B) Dynamic reaction simulation of PcBPGIP (green) inhibiting substrate cleavage by blocking certain residues away from the cleft of *Xf*P.

Objectives 3 and 4
No activity planned for this reporting period.

CONCLUSIONS

1. Fourteen selected PGIPs have been identified that are likely candidates to effectively inhibit *Xf*PG.
2. Cloning has progressed to obtain each of the selected PGIPs in a format so their *in planta* and *in vitro Xf*PG inhibiting activities can be tested.
3. Molecular modeling has progressed so that differences in ability to inhibit *Xf*PG can be related to unique conformational properties of the selected PGIPs.
4. *Xf*PG has been expressed in Drosophila cells to obtain material for *in vitro* analysis of the inhibition activity of the selected PGIPs.
5. Relevant federal agencies have been consulted for regulatory issues related to commercial product development.

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DO CELL WALL STRUCTURES LIMIT *XYLELLA FASTIDIOSA* DISTRIBUTION IN INOCULATED, PIERCE'S DISEASE SUSCEPTIBLE AND RESISTANT GRAPEVINES?

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ABSTRACT
The development and progression of Pierce's disease (PD) symptoms depends largely on the ability of the pathogen to spread via xylem, more specifically, its vessel system, in the infected grapevine. We believe that to the *Xylella fastidiosa* (*Xf)* entering vessels, pit membranes (PM) separating neighboring vessels should become barriers which the pathogen must digest to enhance its systemic spread. Production of occlusions (tyloses and pectin-rich gels) in vessels in response to the presence of *Xf* may also be related to disease symptom development or the host plant's resistance. The research included in this report focuses on these two factors of the host plant which should affect *Xf*'s systemic spread in the host plant. Our data revealed that grape varieties with different PD resistance were different in some cell wall polysaccharides of PMs, that intervessel PMs may be modified in infected PD susceptible grapes and that development of many vessel-obstructing tyloses in response to the presence of *Xf* should contribute to the PD symptom development of the host plant. These observations provide information for understanding of the possible roles of these factors in grape's resistance to PD and are also likely to contribute to identification of an efficient approach for control of the disease.

LAYPERSON SUMMARY
Several of the approaches currently being investigated as strategies for management of Pierce's disease (PD) in vineyards are based on studies that identified the way the disease becomes established in a grapevine. The relative resistance/susceptibility of range of grape genotypes has been studied in the past decade. The work described here asks whether the pathway used by *Xylella fastidiosa* (*Xf)* to spread through a grapevine, the so-called pit membranes (PMs), differ between susceptible and tolerant grape lines. It also asks whether the development of vascular system obstructions, barriers that could either prevent *Xf* spread or shut down vine water transport, or both, differ in susceptible and resistant vines. The data suggest that the polysaccharide compositions of the PMs are different (in terms of kinds or amounts of polymers present) in susceptible and resistant vines. Whether these differences are important in determining whether a given grape germplasm will be PD resistant or tolerant is not yet known.

INTRODUCTION
Pierce's disease (PD) is a devastating grapevine disease caused by the xylem-limited bacterium, *Xylella fastidiosa* (*Xf)*. It is clear that vine death is caused by the systemic spread of the locally introduced *Xf* throughout the vine (Krivanek and Walker, 2005; Labavitch, 2007; Lin, 2005; Lindow, 2006a, b, 2007a, b; Rost and Matthews, 2007). The initial introduction of *Xf* by the glassy-winged sharpshooter (GWSS) involves only in few vessels. To spread throughout the grapevine, *Xf* cells must move successively from one vessel to another. The neighboring vessels are separated from one another by the so-called pit membranes (PMs), primary cell wall "filters." Since the meshwork of PMs is too small to permit *Xf* passage, an increase in PM porosity is a prerequisite for spread of the *Xf* population in a host plant (Labavitch et al., 2004).

Xf's genome contains genes encoding cell wall-degrading enzymes (CWDEs), including polygalacturonase (PG) and a few ß-1,4-endo-glucanases (EGase). We believe that *Xf* cells use the CWDEs to digest the polysaccharides of the PMs, opening the primary cell wall barrier and allowing *Xf* passage. This supposition has been supported by several studies performed over the past several years. Roper et al. (2007) reported the generation of a PG-deficient strain of *Xf* and showed that it was unable to cause PD symptoms, thus identifying the pathogen's PG as a PD virulence factor. Labavitch et al. (2006) reported that introduction of PG and EGase into explanted stems of uninfected grapevines caused the breakage of the PM cell wall network.

Research in the laboratories of the PIs on the present proposal has shown that the substrates for *Xf*'s CDWEs, pectins and xyloglucans, are present in grapevine PMs (Labavitch, 2007; Labavitch and Sun, 2008) and that PG-inhibiting proteins (PGIPs) limit the development of PD in grapevines (Agüero et al., 2005). Research in Cooperator Steve Lindow's program

has focused on the role of a diffusible signal factor produced by *Xf* in controlling the pathogen's expression of virulence functions that affect whether the pathogen spreads systemically in grapevines and causes PD (Lindow, 2007a, b). Cooperator Andy Walker and his colleagues have identified a grapevine QTL that contains the Pierce's disease resistance (*PdR1*) locus (Walker and Riaz, 2007) that eventually will be deployed in grapevine genotypes that will have enhanced resistance to PD. Walker, Lindow and Cooperator Hong Lin have all made use of natural variations in the PD resistance/susceptibility of different grape germplasm in order to understand the factors that influence *Xf* movement in grapevines and, therefore, PD development. It is reasonable to assume that differential PD susceptibility of grape genotypes is determined by (1) genetic variation in PM barriers to pathogen movement that are expressed as differences in porosity, polysaccharide composition or susceptibility to *the pathogen's* CWDEs or/and (2) the post-infection deployment of tyloses and gels, factors that could restrict the pathogen to the few vessels into which it has been introduced.

Grape genotypes show differential PD resistance. Most *vinifera* varieties are susceptible to PD, while wild *Vitis* species and some of their hybrids with *vinifera* varieties have been demonstrated to have PD tolerance or resistance in greenhouse and field evaluations. Quantitative analyses of the concentration and distribution of the pathogen have clarified that *Xf*'s spread from the inoculation site in resistant genotypes is limited relative the its spread in susceptible *vinifera* varieties (Lindow, 2007a), suggesting differences in PM polysaccharide composition among the genotypes with differential PD resistance. Therefore, the clarification of any possible cell wall compositional differences in PMs of those grape varieties/genotypes is essential to the better understanding of the natural PD resistance mechanisms of grapes.

While the production of gels and tyloses in response to infection have been examined in several programs (e.g., Lin, 2005; Stevenson et al., 2004), detailed information about the spatial and temporal distributions and of vascular occlusions in susceptible and resistant germplasm is still lacking. This information is crucial to clarify the role of the vascular occlusions in PD symptom development or disease resistance of host plant. An efficient system to evaluate the development of vascular occlusions in grapevines quantitatively and qualitatively has been developed by Co-PI Sun (Sun et al., 2006, 2007 and 2008) and was used in this study. The utility of immunohistochemical techniques in identifying the polysaccharides of grapevine PMs and vascular occlusions has recently been demonstrated by Co-PI Sun (Labavitch, 2007). These techniques may contribute to an understanding of the differences in xylem water-conducting cell structures that have been thought by many to hold the key to grapevine resistance to PD. This proposal will use these techniques in several grape germplasms where differential resistance to PD has been shown in order to obtain the detailed structural and spatial information that may help explain why some grapevine genotypes are resistant to PD while others are not. These results may provide the information useful for finding an effective approach for control of grape PD.

OBJECTIVES

1. Determine if the development of xylem obstructions (tyloses and pectin-rich gels) and the polysaccharide structure and integrity of pit membranes are affected by *Xf* inoculation of grapevines transformed to express the PGIP from pear and other plant species in rootstocks and in scions.
2. Determine whether there are differences in pit membrane porosity or polysaccharide structure between resistant and susceptible grapevines. To what extent are these PM characteristics and the production of tyloses and gels modified by introduction of *Xf* to PD-resistant and -susceptible genotypes?

   (**Note**: The original proposal had four Objectives, but only Objectives 1 and 2 were approved for funding.)

RESULTS AND DISCUSSION

Differences in cell wall polysaccharide compositions of pit membranes of grapevines with differential PD resistance

In this research, we have used the following grape genotypes/varieties with different PD susceptibility: *Vitis vinifera var.* Chardonnay (susceptible), *Muscadinia rotundifolia* (highly tolerant) and 89-0908 (resistant, a hybrid of *V. arizonica* x *rupestris*). The immunohistochemical techniques and confocal laser scanning microscopy we established previously were used to identify and compare polysaccharide compositions of the vessel PMs in these genotypes/varieties. The research covered both intervessel PMs and vessel-parenchyma PMs, which exist in vessel lateral walls. The former are the barriers to *Xf*'s systemic spread, while the latter are related to the development of vascular occlusions (tyloses and gels) and may contribute to disease resistance or symptom development.

Our experiments focused on two major groups of cell wall polysaccharides: homogalacturonans (the predominant components of pectin) which polygalacturonases may attack, and xyloglucans (XyGs, a major group of hemicellulosic polysaccharides), the substrates of endo-glucanases. We have used three different kinds of monoclonal cell wall antibodies to identify the polysaccharide composition of PMs: JIM5, JIM7 and CCRC-M1. JIM5, JIM7 and CCRC-M1 can recognize weakly methyl-esterified homogalacturonans (low Me- HGs), heavily Me-esterified HGs (high Me-HGs), and fucosylated XyGs, respectively. Our aim is to determine whether there are any differences in the presence or distributions of these two groups of polysaccharides in the PMs of the four genotypes/varieties studied.

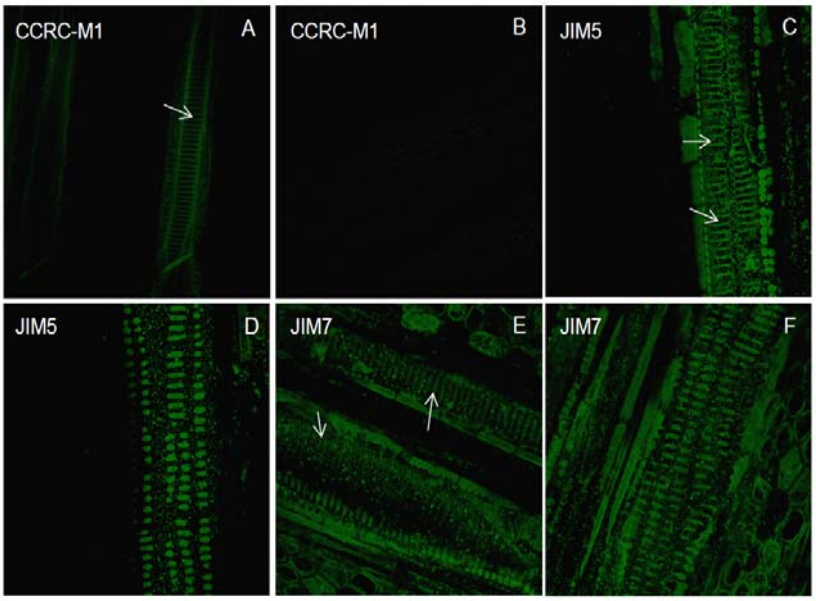

Figure 1. Cell wall compositions in intervessel pit membranes (A, C, E) and vessel-parenchyma PMs (B, D, F) in 89-0908, a PD-resistant *Vitis* genotype. A-B, No green fluorescence from intervessel PMs (A) and vessel-parenchyma PMs (B) in xylem tissue treated with CCRC-M1, indicating that fucosylated XyGs in both types of PMs are below the detectable level. C-D. PM composition revealed by JIM 5. Low Me-HGs are detected in vessel-parenchyma PMs (arrowed, D) but not in intervessel PMs (arrows, C). E-F. PM wall composition revealed by JIM7. Very weak fluorescence and relatively strong fluorescence are detected from intervessel PMs and vessel-parenchyma PMs, respectively, indicating that high Me-HGs are at a low concentration in intervessel PMs but in larger amount in vessel-parenchyma PMs.

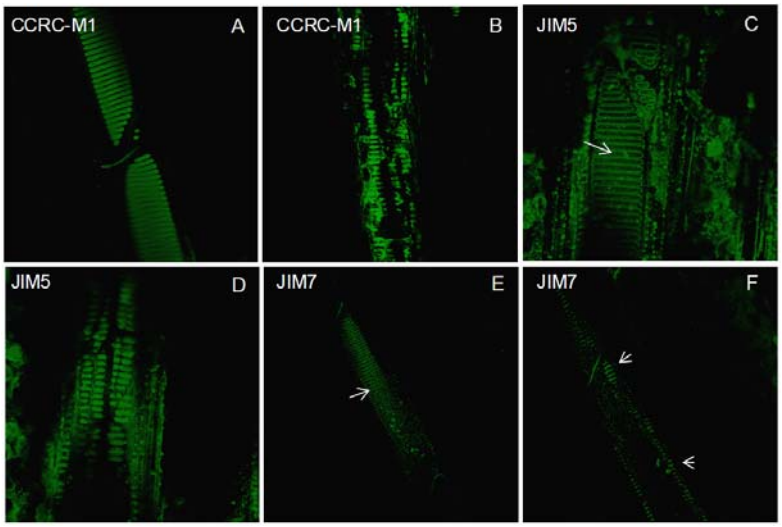

Figure 2. Cell wall compositions in intervessel PMs (A,C,E) and vessel-parenchyma PMs (B,D,F) in *Muscadinia rotundifolia*, a highly PD-tolerant grape genotype. A-B, Cell wall composition revealed by CCRC-M1, showing the presence of fucosylated XyGs in both intervessel PMs (A) and vessel-parenchyma PMs (B). C-D. Cell wall composition revealed by JIM5. Low Me-HGs are not obvious in intervessel PMs (C) but are present abundantly in vessel-parenchyma PMs (D). E-F. Cell wall composition revealed by JIM7. Fluorescence signal is detected from both intervessel PMs and vessel-parenchyma PMs, but is relatively weak, indicating a limited amount of high Me-HGs in both types of PMs.

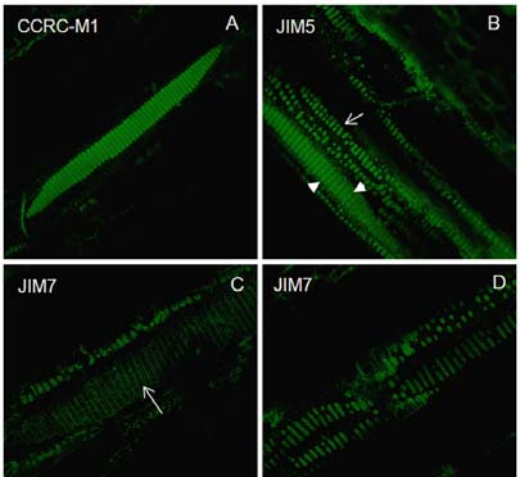

Figure 3. Cell wall compositions of intervessel PMs (A,C,E) and vessel-parenchyma PMs (B,D,F) in *Vitis vinifera* cv. Chardonnay, a PD-susceptible genotype. A. Intervessel PMs have strong fluorescence when incubated with CCRC-M1, indicating the abundant presence of fucosylated XyGs. B. Xylem tissue incubated with JIM5, showing that low Me-HGs are common components of both intervessel PMs (arrow head) and vessel-parenchyma PMs (arrow). C-D. Xylem tissue incubated with JIM7. Fluorescence is below the detectable level in intervessel PMs (arrow, C) and is strong from vessel-parenchyma PMs (D), indicating high Me-HGs is weakly present in intervessel PMs (C) but is abundantly present in vessel-parenchyma PMs (D).

Our results have indicated that the four genotypes with different PD susceptibility all have intervessel PMs and vessel-parenchyma PMs in their vessel lateral walls. Individual intervessel PMs are transversely elongated across the whole surface of the shared (i.e., common) wall of neighboring vessels and are arranged in a tight scalariform pattern along the vessel long axis (Fig. 2A). Vessel parenchyma PMs are round, oval or slightly transversely elongated (**Figure 2D**).

The genotypes also showed differences in the polysaccharide compositions of intervessel and vessel-parenchyma PMs. In 89-0908, both intervessel PMs (**Figure 1A**) and vessel-parenchyma PMs (**Figure 1B**) lack fucosylated XyGs In addition, their intervessel PMs do not have a detectable amount of low Me-HGs (**Figure 1C**) or high Me-HGs (**Figure 1E**). However the vessel-parenchyma PMs contain both low Me-esterified (**Figure 1D**) and high Me- HGs (**Figure 1F**). In *Muscadinia rotundifolia*, strong fluorescence signals were detected from both intervessel PMs (**Figure 2A**) and vessel-parenchyma PMs (**Figure 2B**) when incubated with CCRC-M1 (showing fucosylated XyGs) in both types of PMs. Some high Me-HGs are also present in both types of PMs (**Figures 2E and 2F**). Low Me-HGs occur in vessel-parenchyma PMs (**Figure 2D**) but are not detected in intervessel PMs (**Figure 2C**). In *V. vinifera var.* Chardonnay, fucosylated XyGs (**Figure 3A**) and low Me-HGs (**Figure 3B**) are abundantly present in both intervessel PMs and vessel-parenchyma PMs. High Me-HGs occur in a large quantity in vessel-parenchyma PMs (**Figure 3D**), but are undetectable in intervessel PMs (**Figure 3C**).

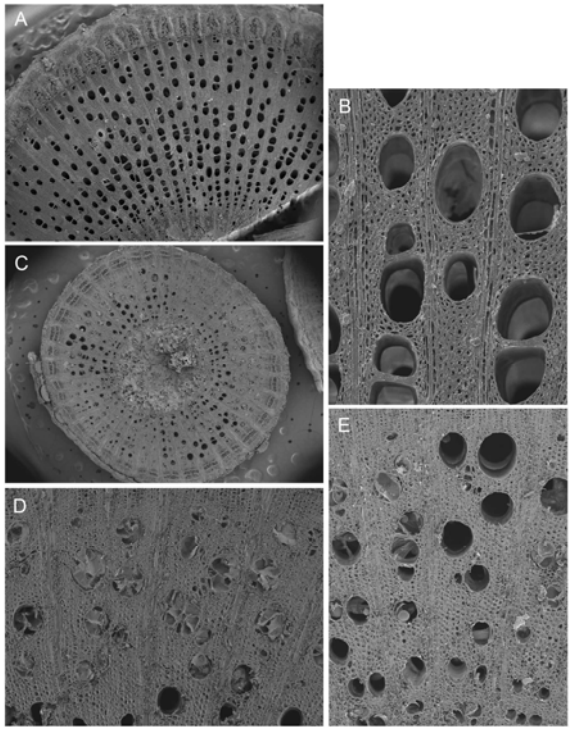

Figure 4. Xylem structure of control (A and B) and inoculated (C-E) vines. A-B. No vascular occlusions occurred in secondary xylem vessels (A); a closer image shows that vessel lumens are empty (B). C. Vascular occlusions developed in secondary xylem of inoculated branches and showed uneven distribution. D. A xylem region with extensive vascular occlusions, showing most vessels blocked by tyloses. E. Xylem region with fewer vascular occlusions and some empty vessels.

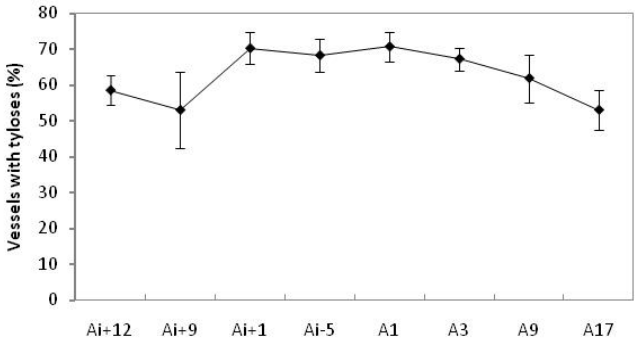

Figure 5. Comparison of vascular occlusion occurrence among different internodes of the two shoots of a same vine. "Ai" and "A" are the shoots with *Xf* inoculation and without inoculation, respectively. The number following "Ai" indicates a specific internode with the positive or negative number showing that the counting of internode started from the inoculated internode and moved upward (positive) or downward (negative), respectively. The number following "A" shows the internode in the non-inoculated shoot, counted from its base.

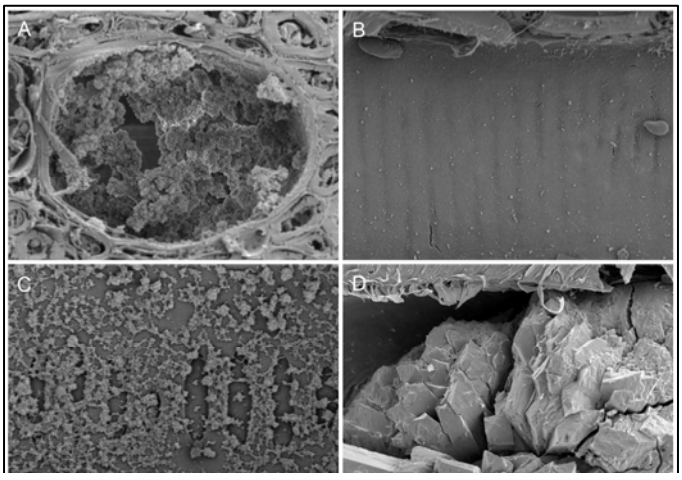

Figure 6. Other types of vascular occlusions in infected grapevines. A. Gels in a vessel lumen. B. Gels covering the lateral wall of a vessel. C. Gels sparsely attached to the vessel lateral walls. D. Crystals filling a vessel lumen.

Comparison of vascular occlusion formation between control vines and sick vines

PD susceptible Chardonnay vines were used in our experiment. Each chardonnay vine on rootstock was pruned back with only two buds left at the base. The two buds thus develop into two branches. When the branches are six weeks-old, one branch of each treatment vine was needle-inoculated with *Xf* at the 12th internode from the base. Vines for controls were inoculated at the corresponding internode with phosphate buffer also on one of the two branches for each vine. Both

branches of each vine (control and treatment) were kept about 25 nodes in height by pruning the top off. Samples were collected from both branches of each vine for both control and treatment vines at different times after the inoculation. Included here are only the data from the vines at Week 12 after inoculation when severe external PD symptoms of the treatment vines have developed.

The vines inoculated with *Xf* and those inoculated with buffer showed obvious differences in secondary xylem structure (**Figure 4**). In control vines, no vascular occlusions were observed in secondary xylem, even in the internode with the inoculation of buffer (**Figures 4A and B**). In vines treated with *Xf*, extensive formation of vascular occlusions occurred in secondary xylem vessels (**Figure 4C**). Vascular occlusions in infected vines were not even in vessels across the transverse section. Instead, in some regions of xylem, they were present in most of the vessels (**Figure 4D**), while in other regions, some vessels were free of vascular occlusions (**Figure 4E**). The cause for patchy occurrence of vascular occlusions in secondary xylem is not known.

Investigation of the spatial distribution of vascular occlusion indicated that it occurred to the internodes of both branches of each infected vine, no matter how far away the internodes were from the inoculation site. Quantitative analysis of vascular occlusions revealed that the percentage of the vessels with one or more vascular occlusions was usually around 60% in all the examined internodes and that no big difference can be distinguished between the two branches of each vine as well as among different internodes of each branch (**Figure 5**).

When tracking through vessels in the longitudinal direction, we found that tyloses did not always continuously block a whole vessel; a given vessel may have some gaps where no occlusions developed. With this in consideration, the actual percentage of vessels affected by vascular occlusions should be higher than the value measured at any transverse section. The effect of vascular occlusion on hydraulic conductivity of xylem is to be evaluated.

Our investigation also clarified that three types of vascular occlusions excluding *Xf* formed in secondary xylem. Tyloses are the predominant type and accounted for over 95% of the occlusions in vessels (**Figures 4C and D**). Pectin-rich gels were another type of occlusion observed; these formed usually in less than 3% of the total vessels (**Figures 6A-C**). Occasionally, crystals were found in the vessels of infected vines and may partially or completely block the affected vessels (**Figure 6 D**).

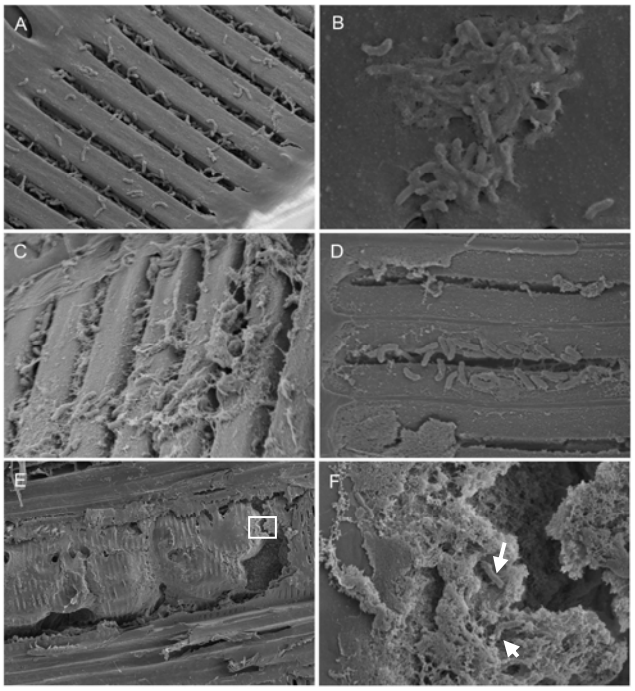

Figure 7. Distribution of *Xf* in infected vines. A. Bacteria are mostly present freely in the internode just above the internode with the inoculation site. B. Many bacteria in an aggregate in the 9th internode (the counting started from the inoculated internode with it as zero). C. Some free bacteria and some bacteria in an aggregate in the 9th internode of the non-inoculated shoot (the counting started from the shoot base with the lowest internode as one). D. Free bacteria in the 17th internode of the non-inoculated shoot (the counting started from the shoot base with the lowest internode as one). E. A vessel filled with tyloses in the lowest internode of the non-inoculated shoot. Gels were present between tyloses. F. Enlargement of the rectangle region in E, showing bacteria embedded in the gels.

Distribution of Xf after inoculation

In the vines with severe external PD symptoms, *Xf* cells were observed in all the examined internodes of the two branches (**Figure 7**). This indicated that the bacteria could move not only upward from the inoculation site in the shoot, but also travelled downward, from the inoculated shoot to the trunk shared by the two branches, and then moved into the non-inoculated branch and travelled up towards its top internodes.

Our observations also indicated that bacteria in the vines with severe external PD symptoms were present in very few vessels. Vessels with *Xf* were usually less than 10% and 3% of all vessels in the inoculated and non-inoculated shoots, respectively. The number of bacteria in the affected vessel was also larger in the internodes of an inoculated shoot than in those of a non-inoculated shoot. However, no vessels with enough bacteria to completely block vessels were observed, as suggested by some earlier studies . Since *Xf* are only present in few vessels in limited amount, a direct influence of bacterial inhabitation on the water transport through the vessel system should be very limited.

Xf were present in vessel lumens in several different forms. Most commonly, they occurred as free individuals (**Figures 7A and D**). Bacteria in this form were observed in the internodes of both inoculated and non-inoculated shoots. Aggregates of 2-6 cells were also common, in which bacteria are loosely bound together through a filamentous network (**Figure 7C**). Occasionally, aggregates formed by tens or hundreds of bacteria were observed in some vessel lumens (**Figure 7B**). Bacteria were also observed between loosely or compactly arranged tyloses (**Figures 7E and F**). In this case, bacteria were always embedded in gels whose origin (tylose or bacterium) is not clear.

CONCLUSIONS

1. Grape varieties/genotypes with differential PD resistance show differences in the cell wall polysaccharide composition of intervessel PMs. The intervessel PMs of resistant genotypes lack fucosylated xyloglucans and weakly Me-esterified HGs, and contain only a little amount of heavily Me-esterified HGs, while the PMs of the more susceptible genotypes/varieties all have fucosylated xyloglucans, and contain substantial amounts of either heavily Me-esterified HGs or weakly Me-esterified HGs. The absence of polysaccharide substrates for *Xf*'s CWDEs in intervessel PMs of resistant genotypes may limit the ability of the pathogen to move away from the inoculation point and, thus, may contribute to the localized distribution of *Xf* in host plant and its PD resistance.
2. Multiple types of vascular occlusions (tyloses, gels and crystals) may develop in infected vines, but tyloses are the principal occlusion type which blocks the majority of vessels, contributing the symptom development.
3. *Xf* may occur in diverse forms (singly, or in groups) and in different parts of the vines with severe PD symptom, but the *Xf* cells are present in only few vessels where they are too low in number to block the vessels.

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SEASONAL TRANSMISSION OF *XYLELLA FASTIDIOSA* BY GLASSY-WINGED SHARPSHOOTER FROM GRAPEVINES: SHARPSHOOTER PREFERENCE FOR INFECTED GRAPEVINE TISSUE

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Reporting Period: The results reported here are from work conducted July 2007 through September 2009.

ABSTRACT
This study is part of our larger project aimed at understanding the feeding biology of the glassy-winged sharpshooter (GWSS) as it relates to acquisition and transmission of *Xylella fastidiosa (Xf)*. Over the course of this two year project we have determined that GWSS males and females choose to feed on young leaf, petiole, and stem tissue compared to the same tissues on older parts of the grapevine cane, regardless of the time of year. However, they will feed on old stem tissue, which logically should result in more rapid chronic infection than feeding on young tissue. GWSS adults frequently change position between various tissues through the day, which may contribute to the apparent effectiveness in spreading *Xf*. We have determined that GWSS adults do not feed on cordon tissue, regardless of the time of year. In winter studies, we found that GWSS prefer to feed on grapevine tissue that is infected with *Xf* over tissue that is not infected. This has tremendous implication for bacterial acquisition during the dormant periods of the year, and since GWSS adults retain *Xf* for life, this represents another interesting feature of this invasive vector that may contribute to Pierce's disease (PD) spread. In fall studies when vines were in full flush, the preference for infected tissue was not present. Both GWSS and the closely related smoketree sharpshooter (STSS) fed equally on infected and non-infected grapevine tissue. This work shows yet another aspect of GWSS and STSS biology that is important to the spread of *Xf*.

LAYPERSON SUMMARY
The detailed experiments that have been conducted in this project have tremendous implication for the movement of *Xylella fastidiosa* (*Xf)* by the glassy-winged sharpshooter (GWSS). We have learned that GWSS showed a strong preference for grapevine canes from infected vines in the winter months. This aspect of GWSS biology is interesting and contributes to its status as a vector of *Xf* in grapevines. If we can determine the cause of this preference, we may be able to design methods to reduce it. Studies in the fall months did not reveal a preference for infected or non-infected grapevine tissue. We found that GWSS and smoketree sharpshooter (STSS) move readily between infected and non-infected tissue, again a behavior that would contribute to *Xf* in the field. Studying these detailed behaviors contributes to our understanding of the epidemiology of Pierce's disease vectored by GWSS and STSS.

INTRODUCTION
Pierce's disease (PD), a disease of grapes caused by the bacteria, *Xylella fastidiosa* (*Xf)* Wells et al., was described in California in the 1880s during an epidemic in Orange County (Pierce 1882). A second epidemic occurred in Tulare County in the 1930s (Hewitt et al. 1949), and until the mid-1990s, it was considered only a minor problem in vineyards close to riparian areas. In the early 1990s a new vector, GWSS, was introduced into the state (Sorenson and Gill 1996), and became associated with a devastating epidemic of PD in the Temecula Valley. Since 1994, at least 1,500 acres of vineyards have been lost to the disease in California; in the Temecula Valley alone, losses have been estimated at $13 million (Wine Institute 2002).

The glassy-winged sharpshooter (GWSS) has different feeding and dispersal capabilities than native insect sharpshooter vectors and these attributes are thought to have contributed to the increased number of PD-infected grapevines in California (Almeida et al. 2005a, Blua et al. 1999, Redak et al. 2004). Like other insect-borne plant pathogen systems, there are two potential types of pathogen spread: primary or secondary spread. Primary spread occurs when the pathogen is obtained by the vector from sources outside the crop and transported and inoculated into the crop. Secondary spread occurs when the vector acquires the pathogen from infected vines in the vineyard, and subsequently inoculates healthy vines within the same vineyard (i.e. vine to vine spread). It is thought that *Xf* spread with native California vectors was the result of primary spread, but that rapid spread by GWSS may be the consequence of primary and secondary spread (Almeida et al. 2005a, Hill 2006). GWSS landing and feeding behavior and tissue feeding capacity combine with grapevine phenology, and within-vine *Xf* distribution and phenology to make vine to vine spread possible. Our overall goal is to provide information on these various components to enhance our understanding of vine to vine spread so that strategies can be defined to reduce widespread epidemics in other regions.

We have conducted experiments in the fall, winter, and summer in which we made hourly observations on the location of individual GWSS adults given access to mature tissue and young tissue on the same cane. Both males and females preferred young tissues (particularly the stems) to mature tissues on Cabernet Sauvignon and Chardonnay grapevines throughout the year. However, GWSS spent a substantial amount of time feeding on old stem tissue (7.5%, 11%, 15% in fall, winter, and spring trials, respectively) (Perring et al. 2008), where *Xf* could potentially be transmitted leading to chronic infection. A significant finding is that GWSS moved frequently throughout the days of our studies, changing position in 35%, 14%, and 21% of the observations in the fall, winter and spring, respectively. This has serious consequence for moving *Xf* around the vineyard at various times of the year. Further characterization of GWSS feeding behavior was conducted in no-choice studies. We learned that at no time of the year, were individuals able to feed on the cordon tissue. While others have observed GWSS feeding in this tissue (Almeida et al. 2005b), we were not able to demonstrate it in our trials on mature vines. Aside from cordons, GWSS were able to feed on old and young stems, petioles, and leaves. However, the amount of feeding varied with the season. In the winter and summer, GWSS utilized old stems and young stems, while during the fall they were not able to feed on old stems. In addition, the young stems became hardened and woody, and survival and feeding on the young stems at this time of the year were reduced. Our goal is to integrate the information from these past studies with present and future research on infected grapevines at different times of the year. Through this work, we will understand the interaction between feeding behavior on specific grapevine tissues that contribute to the spread of *Xf* from infected to healthy vines.

OBJECTIVES:

1. Document GWSS feeding preference, through the growing season, on established Cabernet Sauvignon and Chardonnay grapevines that either are healthy or have been infected with *Xf* for two, three, or four years.
2. Evaluate the acquisition by GWSS, through the growing season, from established Cabernet Sauvignon and Chardonnay grapevines that either are healthy or have been infected with *Xf* for two, three, or four years and determine the subsequent transmission from these acquisitions.
3. Determine the relationship between *Xf* inoculation by GWSS at different times of the year and the development of the vine as a source for further acquisition by GWSS.

We were forced to modify the original objectives due to the fact that suspected infections of our grapevines were not present. At the time we started in July 2007, selected vines in our field cages had been needle-inoculated in May 2003, May 2004, and May 2005 by cooperator Groves. An evaluation of all the vines on August 28, 2007 showed almost no infection with *Xf*. It is unclear why the infections did not become systemic, but the fact that we had no multi-year infections dictated a revision of our original plans. We re-inoculated the set of vines that had been inoculated in 2003 by scraping the bark on the cordons to expose green tissue for needle inoculation. This procedure was done on November 5, 2007 and September 8, 2008 and has yielded severe infections for us to use. While waiting for infections, we proceeded with experiments to document GWSS feeding biology through the season in choice and no-choice studies. Below we summarize these studies, the data of which are presented in Perring et al. (2008).

RESULTS AND DISCUSSION

Choice and No-choice Studies

Choice studies were conducted in the fall 2007 (August 29, and September 11, 2007), winter 2008 (January 16, and February 6, 2008) and summer 2008 (July 1, 2008). For this research, we placed GWSS adults individually in observation cages fabricated from acetate cylinders (25cm x 17cm diameter) with organdy sleeves attached to the ends. The cage was placed over the base of a Cabernet Sauvignon or Chardonnay grapevine cane with the cane terminal looped back into the cage. The ends of the observation cage were sealed giving a single GWSS in each cage access to old and young stems, petioles, and leaves inside the cage. We made hourly observations during daylight hours over three consecutive days to determine the location of each GWSS. When given a choice, GWSS males and females chose to feed on young leaf, petiole, and stem tissue compared to the same tissues on older parts of the cane. However, there was substantial time spent feeding on old stem tissue, a phenomenon that would result in more rapid chronic infection than feeding on young tissue. We also learned that

throughout the day, GWSS adults change position frequently between the various tissues, a characteristic that would support the rapid spread of *Xf* that has been associated with GWSS.

No-choice studies were conducted in the winter 2008 (February 26, March 4), summer 2008 (July 15), and fall 2008 (September 19). Individual GWSS were caged on selected grapevine tissue in 50 ml polypropylene centrifuge tubes by one of two methods. The first method, modified from Andersen et al. (1992), was for use on cordons, stems, and petioles. The cages were made by melting a transverse hole in the side of the tube using hot metal cylinders of diameters similar to the grape tissues. The tube was pressed onto the plant tissue, so the GWSS had access to about 2.5 cm length of the plant through the hole. The cage was affixed and sealed to the tissue by wrapping the tube and tissue with ca. 2 cm wide strips of Parafilm. The screw cap was tightened, and the cage rested vertically so that excreta collected in the bottom of the tube. The second cage design was for use on leaf tissue. The mouth of an intact 50 ml tube was pressed to the abaxial leaf surface with a piece of coiled spring steel in a clothes-pin like fashion (Blua and Perring 1992). One end of the spring held the 50 ml tube. The other end of the spring had a plastic ring on which was glued a foam pad 1 cm thick by 3 cm in diameter which gently held the leaf against the polypropylene tube, giving the insect access to leaf tissue of ca. 5.7 cm^2. This cage, too, was oriented vertically, so excreta drained to the bottom of the cage. Each cage type was loosely covered with aluminum foil in order to shade it from direct sunlight. The day before the start of each test, GWSS adults were collected from citrus at Agricultural Operations, UCR, and placed in a cage with a potted rough lemon plant. The following morning, adults were isolated and sexed and then placed individually into the tube cages. Cages were inspected daily and the presence of excreta noted; cages with dead GWSS were removed, and the amount of excreta was weighed. The sharpshooters were allowed to feed for four days.

In these studies, we found that GWSS adults were not able to feed on cordon tissue, regardless of the time of year. They were able to feed on old and young grapevine tissue throughout the year, but the relative amount of feeding on this tissue varied with the season.

GWSS preference for infected/non-infected grapevine tissue

We selected canes from putative infected and non-infected Cabernet Sauvignon and Chardonnay grapevines to study GWSS choice for infected or non-infected tissue. Trials were conducted with GWSS on February 19-21 and February 25-27 2009. Because of the time of year, there were no leaves or petioles on the canes. All tissue had a brown hardened outward appearance, but we confirmed that the internal tissue was green, so GWSS would be able to feed. We placed GWSS adults individually in observation cages, which were placed over a section of cane from an infected vine and a section of cane from a non-infected vine (**Figure 1**). The infected cane was marked with a small wire label. All sharpshooters were placed on the cage, so they were forced to make a choice to find a feeding host. The ends of the observation cage were sealed giving a single GWSS in each cage access to infected or non-infected cane tissue. Twenty cages were used for each trial. We made hourly observations from 8am to 5pm over three consecutive days to document the cane (infected or non-infected) on which the GWSS fed.

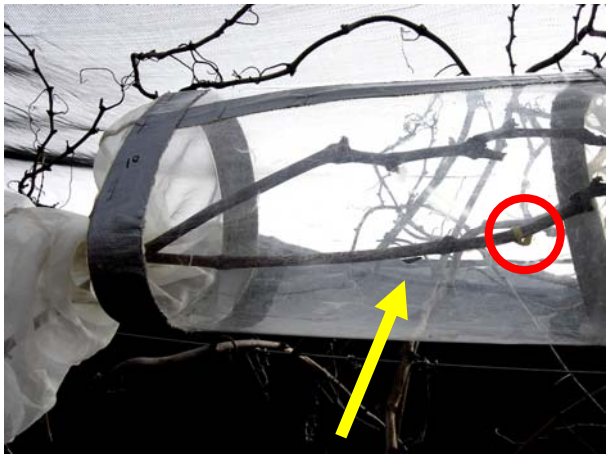

Figure 1. Acetate cage uses to evaluate GWSS feeding preference for infected (marked with yellow wire label (in circle) and non-infected grapevine tissue. Notice GWSS feeding in center of infected cane (arrow).

At the conclusion of the studies, we conducted a variety of procedures to verify the infection status of the cane tissue to which the GWSS were exposed. First each section of both canes that were inside the acetate cages was removed from the vine and a small section (0.5 in) was macerated and subjected to ELISA immediately after the trial was concluded. Second, the cane sections were marked and planted into pots. Following growth of these cuttings, we conducted ELISA and culturing to determine the infection status of the section of cane to which GWSS was exposed. Third, when we pruned the vines, we selected six canes and planted an approximately 14 inch section from each cane into pots to grow in the greenhouse. After they pushed leaves, we assayed these plants by ELISA. Finally, each vine was visually assessed in the fall for symptoms of *Xf* infection. Symptomatic canes were sampled and subjected to ELISA.

From the various tests, we determined the infection status of all the canes used in the experiments and discarded the cages in which we were unable to make a confident determination. We also discarded cages in which the GWSS died, because this indicated the inability of the insect to successfully feed on either cane. This filtering resulted in nine total cages for the February 19-21 trial (four Cabernet Sauvignon, five Chardonnay, four females and five males) and a total of 11 cages for the February 25-27 trial (four Cabernet Sauvignon, seven Chardonnay, six females, and five males). Because of the small numbers present in each variety and gender, the data are presented as totals for each trial.

Results from both trials showed that GWSS was found more often on the infected vines (**Figure 2**). For the February 19-21 test, GWSS were present on the infected tissue 71% of observed times, while they were on non-infected tissue just 22% of the time. They were found on the cage only 7% of the time. In the second trial (February 25-27), they again were found more often on the infected cane (71%) compared to the non-infected cane (22%) or the cage (7%). We were surprised that the proportions for each of these trials were the same, and have no explanation for this similarity. This is particularly remarkable, given that there was a total of 215 observation times in the first trial and 303 observation times in the second trial (**Table 1**) and the two trials were conducted with different insects on different canes, often from different vines, and at two distinct times.

Also interesting were the movements that sharpshooters made throughout the studies. More GWSS moved to infected canes and stayed for three or more hours than to non-infected canes (**Table 1**). Additionally, there were more sharpshooters that fed on infected canes, left these canes and returned to the infected canes, than those on non-infected canes. Clearly there was something unique about the infected canes that the sharpshooters preferred. It also is apparent that sharpshooters in this study moved about the cages often (17 of a possible 215 observations in trial 1 (8%) and 36 of a possible 303 observations (12%) in trial 2).

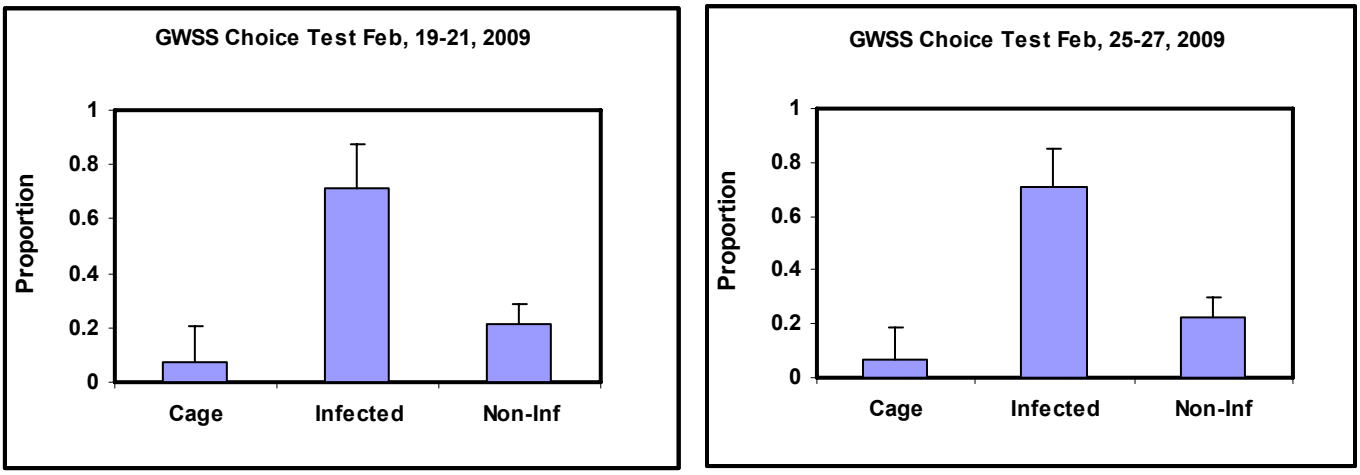

Figure 2. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in choice experiments initiated on 19 February (left) and 25 February (right), 2009. Bars represent average proportions of GWSS (± SE) observed on the cage, on the infected canes, and on the non-infected canes.

Table 1. Actions taken by GWSS in two trials (February 19-21 and February 25-27, 2009). Sharpshooters were given a choice between infected and non-infected cane tissue over the 3 day period and observations were made hourly during the daylight hours.

Parameter	Trial 1 (Feb 19-21)	Trial 2 (Feb 25-27)
Chose Infected and stayed 3h or more	12	13
Chose Non-Infected and stayed 3h or more	3	3
Chose Inf. for 3h, left, returned for 3h or more	3	4
Chose Non-I for 3h, left, returned for 3h or more	0	0
Moved from Cage to Inf.	7	12
Moved from Cage to Non-I	1	7
Moved from Inf. to Cage	5	5
Moved from Non-I to Cage	1	4
Moved from Inf. to Non-I	1	4
Moved from Non-I to Inf.	2	4
Total number of Times insect moved	17	36
Total number of Observed Times	215	303

A second set of choice experiments was conducted in September, 2009. These studies, which had the same design as those conducted in February, utilized infected canes that were severely diseased. A healthy, asymptomatic cane was paired with each diseased cane and the canes were stripped of all but 1 leaf within the experimental cage. Twenty cages were established on Chardonnay vines on September 17, and into each cage we introduced a single GWSS female. Observations were made hourly from 8am to 6pm for three days. Utilizing the same 20 cages on the same canes, a second trial was initiated on September 20 with 20 female smoketree sharpshooters (STSS). Observations again were made each hour from 8am to 6pm for a period of three days.

Sharpshooter responses from these trials were distinctly different from the studies conducted in February. In the September 17-19 trial, a slightly higher proportion of GWSS were observed on the non-infected cane (56%) than on the infected canes (40%), with just 4% of the observations on the cage (**Figure 3**). Interestingly, similar results were found for the STSS. This species showed a slight preference for the non-infected canes (51%) rather than the infected canes (40%), with 9% of the observations on the cage (**Figure 3**). We will be collecting the canes from this study in an effort to analyze the xylem sap to see if any particular chemical constituents were present in the canes on which sharpshooters predominantly fed.

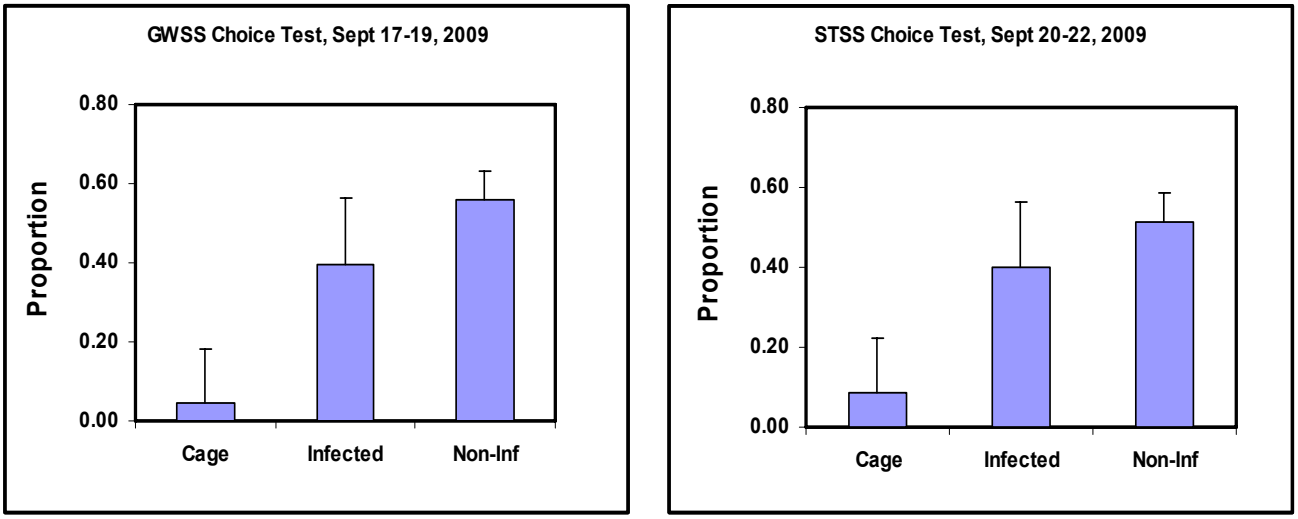

Figure 3. Female GWSS preference (left graph) and STSS preference (right graph) on field-grown Chardonnay grapevines in choice experiments initiated on September 17 (STSS) and September 20 (STSS). Bars represent average proportions of sharpshooters (± SE) observed on the cage, on the infected canes, and on the non-infected canes.

Table 2. Actions chosen by GWSS (September 17-19) and STSS (September 25-27) in choice studies between infected and non-infected cane tissue over the three day period . Observations were made between 8am and 6pm.

Parameter	GWSS (Sept. 17-19)	STSS (Sept. 25-27)
Chose Infected and stayed 3h or more	11	21
Chose Non-Infected and stayed 3h or more	19	25
Chose Inf. for 3h, left, returned for 3h or more	3	9
Chose Non-I for 3h, left, returned for 3h or more	10	5
Moved from Cage to Inf.	15	23
Moved from Cage to Non-I	14	26
Moved from Inf. to Cage	3	17
Moved from Non-I to Cage	10	14
Moved from Inf. to Non-I	12	10
Moved from Non-I to Inf.	8	12
Total number of Time insect moved	62	102
Total number of Observed Times	485	633

Sharpshooters moved slightly more often in this set of experiments than in the February study. In the GWSS trial, insects moved 62 out of a possible 485 observations (13%) and 102 out of 633 observations (16%) (**Table 2**). There were more GWSS that settled and had prolonged feeding (at least 3 hr) on non-infected canes than on infected canes. There was only a slightly higher number of STSS that had prolonged feeding on the non-infected canes than the infected canes.

CONCLUSIONS

In winter choice studies in which GWSS were given access to infected and non-infected grapevine tissue, GWSS were found more often on grapevine tissue that was infected with *Xf* over tissue that was not infected. The reason why this choice was made is unknown, but likely is related to the biochemical components in the various cane tissues (Anderson et al. 1992). Regardless, the fact that GWSS prefers infected tissue has important epidemiological ramifications. Specifically, feeding on infected tissue increases the likelihood of sharpshooters acquiring *Xf*. Movement by these sharpshooters to non-infected tissue, which occurred 1/17 (6%) and 4/36 (11%) times in the two winter trials, could rapidly move the bacteria causing new infections. It is important to remember that the cane tissue was woody (although green inside) and sharpshooters easily fed on this tissue.

In fall experiments, on vines containing green leaves, sharpshooter preference for infected tissue was not apparent. Both GWSS and STSS fed equally on infected and non-infected grapevine tissue. These data suggest that there was nothing in either infected or non-infected tissue that caused sharpshooters to feed preferentially. Both insect species moved readily from infected to non-infected tissue (12/62 = 19% and 10/102 = 10% for GWSS and STSS, respectively). They also moved from non-infected tissue to infected tissue with similar frequency. These results suggest that transmission between infected and healthy vines may be greater at this time of year.

The work reported here is valuable to our understanding of GWSS and STSS feeding behavior that can influence transmission of *Xf*. These studies fill an important data gap in our knowledge of GWSS- and STSS-vectored epidemiology at various times of the year. We plan to continue studies through next year, to confirm the preference of sharpshooters for infected tissue. During this work, we will conduct biochemical assays similar to Andersen et al. (1992) to determine what components are correlated with GWSS and STSS feeding.

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XYLELLA FASTIDIOSA TRANSMISSION BY GLASSY-WINGED SHARPSHOOTERS AND SMOKETREE SHARPSHOOTERS FROM ALTERNATE HOSTS TO GRAPEVINES

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Reporting Period: The results reported here are from work conducted July 2007 to September 2009.

ABSTRACT
This project is designed to evaluate the importance of many common weed, agricultural, and cover crop plants that are found in close proximity to vineyards as sources of *Xylella fastidiosa (Xf)* from which glassy-winged (GWSS) and smoketree (STSS) sharpshooters can acquire and transmit *Xf* into grapevines. In our studies *Xf* was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, annual bluegrass, cheeseweed, wild-type sunflower, goosefoot, London rocket, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand White clover, Hykon Rose clover, cowpea, fava bean, Miranda field pea, meadow barley, California Red oats, and White sweetclover. We were unable to recover *Xf* from bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, or sudangrass plants. We have confirmed successful transmission of *Xf* by GWSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, basil-to-basil, basil-to-grapevine, tomato-to-tomato, Blando brome-to-Blando brome, Blando brome-to-grapevine and cowpea-to-cowpea. GWSS transmission of *Xf* from tomato-to-grapevine, cowpea-to-grapevine, fava bean-to-fava bean, and fava bean-to-grapevine could not be confirmed with culturing. We have determined that STSS can transmit *Xf* between alfalfa plants, from alfalfa to grapevines, between Blando brome plants, from Blando brome to grapevines, and between fava bean plants. We were unable to confirm successful transmission by STSS from tomato-to-tomato, tomato-to-grapevine, cowpea-to-cowpea, or cowpea-to-grapevine, or from fava bean to grapevines. Goosefoot appears to be a poor host for GWSS, STSS, and *Xf*, as nearly all the vectors died before the end of the 48-hr acquisition access period. *Xf* isolates obtained from goosefoot were few and slow-growing.

LAYPERSON SUMMARY
Evaluating the potential of various common plant species found in and near vineyards to serve as reservoirs of Pierce's disease (PD), and the ability of glassy-winged (GWSS) and smoketree (STSS) sharpshooters to acquire and transmit PD from these alternative plant hosts, is fundamental to managing the primary spread of PD in California vineyards. Identifying the plants that contribute to primary spread enables growers to target these plants around their vineyards as a mechanism to reduce spread. Understanding how these two vectors contribute to primary and secondary spread can assist in the development of alternatives to the area-wide management program. To reduce primary spread, efforts must focus on reducing bacteria-carrying vectors from entering healthy vineyards through continued area-wide or local treatment programs outside the vineyard, barriers, trap crops, and/or removal of pathogen sources outside the vineyard.

INTRODUCTION
Over 140 plants are known to host Pierce's disease (PD) strains of *Xylella fastidiosa (Xf)* (Costa et al. 2004, Freitag 1951, Raju et al. 1980, 1983, Shapland et al. 2006, Wistrom and Purcell 2005, http://www.cnr.berkeley.edu/xylella/temp/hosts.htm). Many of these plants are found in close proximity to vineyards, and some are even used as cover crops in vineyards (Statewide IPM Program 2007). While considerable research has identified *Xf* hosts, little work has been done to determine if sharpshooters can acquire the bacteria from these hosts and transmit it to grapevines. If this does not occur, then the alternate host is of little consequence in PD epidemiology. Conversely, plants that contribute inoculum for sharpshooter acquisition and transmission to grape should be removed if growers wish to reduce primary spread into their vineyards.

To successfully implement a program to remove pathogen sources, we first must identify those sources. The introduction into California of the glassy-winged sharpshooter (GWSS), an insect with a broad host range, theoretically increases the probability of disease spread from the these alternate host plants to grape. For this to occur, GWSS must feed on the infected plant in such a way to acquire *Xf* from plant, and successfully transmit the acquired pathogen to grapevines. While studies have shown mechanical and insect transmission to a wide variety of alternate hosts (Freitag 1951, Purcell and Saunders 1999) they have demonstrated transmission from only a handful of alternate hosts to grapevines (Hill and Purcell 1995, 1997). We are unaware of research published on transmission of *Xf*, PD strain, from alternate hosts into grapevines using GWSS or STSS, a native California sharpshooter also found in grape growing regions, as the vector.

OBJECTIVES

Using GWSS and STSS vectors:

1. Evaluate the acquisition and transmission of *Xf* to grapevines from agricultural crop plants known to be PD hosts that are grown in the vicinity of vineyards.
2. Evaluate the acquisition and transmission of *Xf* to grapevines from weed plants known to be PD hosts that are grown in the vicinity of vineyards.
3. Evaluate the acquisition and transmission of *Xf* to grapevines from vineyard cover crop plants.

RESULTS AND DISCUSSION

Needle Inoculated Plants

Thirty-four plant species have been needle-inoculated with *Xf* (**Table 1**). *Xf* does not appear to be able to survive in bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, or sudangrass plants. A few positives were detected at two-weeks post-inoculation with ELISA for bell pepper, cotton, common groundsel, and horseweed, but no plants tested positive by ELISA at four weeks, nor were they positive by culturing. These results suggest a transient infection or detection of dead *Xf* cells by the early ELISA. Evening Sun Sunflower tested positive by ELISA for all 20 plants, but the cultures were clean and negative. However, the Evening Sun sunflower also died very quickly, which may explain why it was not detected by culture. Final results are pending for filaree, Shepherd's purse, and stinging nettle.

Xf was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, annual bluegrass, cheeseweed, wild-type sunflower, London rocket, goosefoot, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand White clover, Hykon Rose clover, cowpea, fava bean, Miranda field pea, meadow barley, California Red oats, and White sweetclover. We recovered one isolate of *Xf* for lima bean in the first needle-inoculation set, so we repeated this test. No isolates were recovered from a second needle-inoculated set, suggesting that lima bean is a poor host for *Xf*. We did not isolate *Xf* from basil until 16 weeks post-inoculation. All ELISA tests for Basil were positive, including those for the negative controls, indicating that the commercial kit for *Xf* from Agdia, Inc. is not reliable for testing this plant species. The cultures for the negative controls were always negative, including at 16-weeks post-inoculation when the positive cultures from other plants were obtained. We only recovered one isolate from annual bluegrass. Cultures from annual bluegrass and fava bean have been routinely heavily contaminated, regardless of plant age or inoculation status. Other microbes present in the plants may be obscuring the presence of *Xf* in those species. Healthy grapevines also were needle inoculated with every inoculation group as positive controls for each set.

Table 1. ELISA and culture results for plant species needle-inoculated with *Xf*.

Type	Common Name	Scientific Name	ELISA +	Culture +	*Xf* Recovered?
Agriculture Crops	Alfalfa	*Medicago sativa*	20/20	14/20	Yes
	Basil, Italian Large Leaf	*Ocimum basilicum*	20/20*	10/20	Yes
	Bell Pepper, Taurus	*Capsicum annuum*	5/20**	0/20	No
	Cotton, Upland	*Gossypium hirsutum*	2/15**	0/15	No
	Lima Bean, Fordhook 242	*Phaseolus lunatus*	2/38	1/38	Yes
	Tomato, Rutgers	*Solanum lycopersicum*	15/39	8/38	Yes
Weeds	Annual Bluegrass	*Poa annua*	8/20	1/20****	Yes
	Black Nightshade	*Solanum nigrum*	0/20	0/20	No
	Cheeseweed	*Malva parviflora*	7/20	16/20	Yes
	Common Groundsel	*Senecio vulgaris*	3/20**	0/20	No
	Common Sunflower, Evening Sun	*Helianthus annuus*	20/20*	0/20	No
	Common Sunflower, wild-type	*Helianthus annus*	19/20	7/20	Yes
	Filaree	*Erodium species*	Tests in Progress		
	Goosefoot	*Chenopodium species*	7/40***	5/33	Yes
	Horseweed	*Conyza Canadensis*	2/20**	0/20	No
	London Rocket	*Sisymbrium irio*	5/20	13/20	Yes
	Shepherd's Purse	*Capsell bursa-pastoris*	Tests in Progress		
	Spanish Broom	*Spartium junceum*	17/20	17/20	Yes
	Stinging Nettle	*Urtica species*	Tests in Progress		
	Tree Tobacco	*Nicotiana species*	12/20**	2/20	Yes

Table 1. ELISA and culture results for plant species needle-inoculated with *Xf* (continued).

Type	Common Name	Scientific Name	ELISA +	Culture +	*Xf* Recovered?
Cover Crops	Annual Ryegrass	*Festuca species*	6/20	6/20	Yes
	Annual Fescue, Zorro	*Lolium multiflorum*	0/20	0/20	No
	Black Mustard	*Brassica nigra*	17/20	13/20	Yes
	Blando Brome	*Bromus hordeaceus*	16/20	13/20	Yes
	Birdsfoot Trefoil	*Lotus species*	10/20	0/20	No
	Clover, New Zealand White	*Trifolium repens*	15/20	2/20	Yes
	Clover, Hykon Rose	*Trifolium hirtum*	16/20	10/20	Yes
	Cowpea, California Blackeye	*Vigna unguiculata*	22/40	16/35	Yes
	Fava Bean, Windsor	*Vicia faba*	30/40	7/20****	Yes
	Field Pea, Miranda	*Pisum sativum*	14/39	3/11	Yes
	Meadow Barley	*Hordeum brachyantherum*	9/20	4/20	Yes
	Oat, California Red	*Avena sativa*	12/20	2/20	Yes
	Sudangrass	*Sorghum bicolor var. sudanense*	0/20	0/20	No
	Sweetclover, White	*Melilotus alba*	20/20	16/20	Yes

* False positives
** Most or all positives in 2-week ELISA test; possible transient infection or dead cells detected.
*** Very slow-growing Xf, detected well after 4-weeks.
**** Fava bean contains many other microorganisms that contaminate and probably obscure positive culture results. Also, fava bean occasionally produces false positives by ELISA.

Insect Transmission

Last year we lost our clean, captive-reared GWSS and STSS colonies to infestations of the parasitoid wasp, *Gonatocerus ashmeadi.* Several of the needle-inoculated plant species died before we were able to rebuild our colonies and perform transmission with them. Therefore, we re-grew and needle-inoculated new sets of those alternative host plants to use for transmission after the colonies sufficiently recovered. To date, transmission using both vector species has been completed for alfalfa, basil, tomato, annual bluegrass, cheeseweed, wild-type sunflower, goosefoot, London rocket, tree tobacco, annual ryegrass, Blando brome, cowpea, fava bean, California Red oats, and White sweetclover. Although transmission has been completed, we are still evaluating the test plants for basil, annual bluegrass, cheeseweed, wild-type sunflower, London rocket, annual ryegrass, California red oats, and White sweetclover, and final data are still pending. At the time of preparing this report, transmission was underway for Spanish broom, black mustard, New Zealand White clover, Hykon Rose clover, Miranda field pea, and meadow barley, with the final data expected to be available in four-eight weeks.

We have confirmed (by culture) successful transmission of *Xf* by GWSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, basil-to-basil, basil-to-grapevine, tomato-to-tomato, Blando brome-to-Blando brome, Blando brome-to-grapevine and cowpea-to-cowpea (**Table 2**). GWSS transmission of *Xf* from tomato-to-grapevine, cowpea-to-grapevine, fava bean-to-fava bean, and fava bean-to-grapevine tested negative by culturing. Only four of 24 GWSS survived the 48-hr acquisition access period (AAP) on goosefoot. The surviving four insects were placed on a clean grapevine test plant, although they appeared to be in the process of dying.

We confirmed successful transmission of *Xf* by STSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, Blando brome-to-Blando brome, Blando brome-to-grapevine and fava bean-to-fava bean (**Table 2**). We were unable to confirm successful transmission by STSS from tomato-to-tomato, tomato-to-grapevine, cowpea-to-cowpea, or cowpea-to-grapevine. All 36 STSS died on goosefoot before the end of the 48-hr acquisition access period (AAP), indicating that goosefoot is a poor host for STSS. Goosefoot also appears to be a poor host for *Xf*, as few cultures were obtained from needle-inoculated plants, and all were extremely slow growing, except for one.

Table 2. Results for transmission of *Xylella fastidiosa* by GWSS and STSS to date.

			GWSS		STSS	
Host Plant Type	PD Acquisition Host	PD Inoculation Host	ELISA +	Culture +	ELISA +	Culture +
Agriculture Crop	Alfalfa	Alfalfa	4/5	4/5	5/5	3/5
	Alfalfa	Grapevine	4/5	4/5	4/5	4/5
	Basil	Basil	9/9	9/9	Tests in Progress	
	Basil	Grapevine	8/9	8/9	Tests in Progress	
	Tomato, Rutgers	Tomato, Rutgers	3/5	1/5	1/5	0/5
	Tomato, Rutgers	Grapevine	2/5	0/5	3/5	0/5

Table 2. Results for transmission of *Xylella fastidiosa* by GWSS and STSS to date (continued).

			GWSS		STSS	
Host Plant Type	PD Acquisition Host	PD Inoculation Host	ELISA +	Culture +	ELISA +	Culture +
Weed	Annual Bluegrass	Annual Bluegrass	Tests in Progress		Tests in Progress	
	Annual Bluegrass	Grapevine	Tests in Progress		Tests in Progress	
	Cheeseweed	Cheeseweed	Tests in Progress		Tests in Progress	
	Cheeseweed	Grapevine	Tests in Progress		Tests in Progress	
	Common Sunflower, wild-type	Common Sunflower, wild-type	Tests in Progress		Tests in Progress	
	Common Sunflower, wild-type	Grapevine	Tests in Progress		Tests in Progress	
	Goosefoot	Goosefoot	0/0	0/0	0/0	0/0
	Goosefoot	Grapevine	0/1	0/1	0/0	0/0
	London Rocket	London Rocket	Tests in Progress		Tests in Progress	
	London Rocket	Grapevine	Tests in Progress		Tests in Progress	
	Spanish Broom	Spanish Broom	Tests in Progress		Tests in Progress	
	Spanish Broom	Grapevine	Tests in Progress		Tests in Progress	
	Tree Tobacco	Tree Tobacco	Tests in Progress		Tests in Progress	
	Tree Tobacco	Grapevine	Tests in Progress		Tests in Progress	
Cover Crop	Annual Ryegrass	Annual Ryegrass	Tests in Progress		Tests in Progress	
	Annual Ryegrass	Grapevine	Tests in Progress		Tests in Progress	
	Black Mustard	Black Mustard	Tests in Progress		Tests in Progress	
	Black Mustard	Grapevine	Tests in Progress		Tests in Progress	
	Blando Brome	Blando Brome	1/4	1/4	4/4	3/4
	Blando Brome	Grapevine	2/4	1/4	0/4	1/4
	Clover, New Zealand White	Clover, New Zealand White	Tests in Progress		Tests in Progress	
	Clover, New Zealand White	Grapevine	Tests in Progress		Tests in Progress	
	Clover, Hykon Rose	Clover, Hykon Rose	Tests in Progress		Tests in Progress	
	Clover, Hykon Rose	Grapevine	Tests in Progress		Tests in Progress	
	Cowpea, California Blackeye	Cowpea, California Blackeye	4/5	2/5	5/5	0/5
	Cowpea, California Blackeye	Grapevine	3/5	0/5	2/5	0/5
	Fava Bean, Windsor	Fava Bean, Windsor	2/5	0/5	1/5	1/5
	Fava Bean, Windsor	Grapevine	1/5	0/5	4/5	0/5
	Field Pea, Miranda	Field Pea, Miranda	Tests in Progress		Tests in Progress	
	Field Pea, Miranda	Grapevine	Tests in Progress		Tests in Progress	
	Meadow Barley	Meadow Barley	Tests in Progress		Tests in Progress	
	Meadow Barley	Grapevine	Tests in Progress		Tests in Progress	
	Oat, California Red	Oat, California Red	Tests in Progress		Tests in Progress	
	Oat, California Red	Grapevine	Tests in Progress		Tests in Progress	
	Sweetclover, White	Sweetclover, White	Tests in Progress		Tests in Progress	
	Sweetclover, White	Grapevine	Tests in Progress		Tests in Progress	

CONCLUSIONS

Bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, and sudangrass did not sustain infection after needle-inoculation with *Xf*, indicating that these plants are very unlikely to harbor *Xf* infection in the field. This is particularly good news for horseweed since it is an extremely common weed in vineyards and is reported to be resistant to herbicides. In their PD management program, growers can choose to target weeds other than those identified here, knowing that these species do not sustain infection with *Xf*. In addition, growers can safely select

Zorro annual fescue, birdsfoot trefoil, and sudangrass as cover crops with confidence that their choice will not contribute to PD spread in their vineyards.

We recovered *Xf* from at least 50% of test plants for alfalfa, basil, cheeseweed, London rocket, Spanish broom, black mustard, Blando brome, Hykon Rose clover, and White sweetclover, indicating that these can serve as hosts for *Xf* in the field. We obtained isolates from three-46% of needle-inoculated plants for lima bean, tomato, wild-type sunflower, goosefoot, tree tobacco, annual ryegrass, New Zealand White clover, cowpea, fava bean, Miranda field pea, and meadow barley. The results from the transmission studies using these plants (pending) should provide a better understanding of their potential as alternative hosts for *Xf* in the field, since needle-inoculation is a severe and unnatural form of infection that is unlikely to happen in the field. As in the case of the goosefoot, we found that we could obtain isolates from a needle-

inoculated plant, but that it was a poor host overall for PD and both vectors tested. Therefore, goosefoot is unlikely to serve as a source or reservoir of *Xf* in the field. If these plants have natural defenses against acquiring or sustaining a *Xf* infection when needle-inoculated with millions of bacteria, it is likely that an infection by a vector transmitting far fewer bacterial cells would be sustained. However, there are insect-pathogen-plant interactions involved that must be tested before such a conclusion can be made definitively. Further studies mimicking more natural acquisition and transmission using insects should be done for a more complete understanding of the roles each plant and vector species might play in the field.

Alfalfa and Blando brome are good hosts for *Xf*, GWSS, and STSS, indicating that they can serve as a reservoir of *Xf* and source of infection in the field for these vectors. Both GWSS and STSS successfully transmitted *Xf* between alfalfa plants, between Blando brome plants, from alfalfa into grapevines, and from Blando brome into grapevines. These two plant species (one a crop plant and the other a cover crop) should not be around or in vineyards where *Xf* or sharpshooters are present.

GWSS successfully transmitted *Xf* between basil plants, from basil to grapevines, between cowpea plants, between tomato plants, and from fava bean to grapevines, but not between fava bean plants, or from cowpea to grapevines. STSS also successfully transmitted between fava bean plants, but not from fava bean into grapevines, between cowpeas, or from cowpea into grapevines. It is possible that *Xf* isolates were obscured by other microbes present in the plants and on the media plates (cowpea and fava bean contain numerous other microbes that grow on media plates for PD), and positive transmission occurred, but was not detected. It also is possible that these plant species would not naturally serve as acquisition sources by these vectors, but because they were unnaturally needle-inoculated, some transmission did occur. Pending further results, careful consideration should be applied when using cowpea or fava bean as cover crops in vineyard areas with known *Xf* infection, or sharpshooter populations, since they can serve as sources. In the unlikely event that basil is grown near vineyards, it could may be a major contributor to the spread of PD, since both GWSS and STSS favor this host, and it could sustain high populations of vectors and harbor *Xf*. The final transmission results (pending) will provide a better understanding of which plant hosts, in combination with GWSS or STSS vectors, are more important in the epidemiology of this plant pathogen.

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BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES AND THE DEVELOPMENT OF MARKERS FOR ADDITIONAL SOURCES OF RESISTANCE

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ABSTRACT
Twenty-two seedless x seedless crosses to develop additional BC2 and BC3 *V. arizonica* and BC1 SEUS BD5-117 families were made in 2008. Powdery mildew resistance was included in five of these crosses. These crosses produced 5,148 berries, 8,824 ovules and 1,841 embryos. Nine seeded BC1 crosses based on *V. arizonica* and SEUS PD resistance sources were made, resulting in 1,393 seed. Two BC2 and 12 BC3 families *(V. arizonica* source of resistance) consisting of 1,191 individuals were screened at the seedling stage in the greenhouse with SSR markers for resistance. A total of 363 were resistant and planted in the field. In November, seedlings from 12 crosses made in 2008 were tested with molecular markers and 159 resistant plants identified from 319 individuals. Greenhouse screening was completed on 150 selections and 63 of the 64 resistant individuals were from *V. arizonica*. Twelve resistant selections have been planted in the field at Weslaco, Texas to determine their field resistance. An additional 89 plants and 692 embryos have been produced to increase the size of the C33-30 x BD5-117 family for molecular maker development. A total of 105 SSR primers are polymorphic between the parents and screening of the first 154 individuals to develop a framework map has started. Greenhouse testing of 125 individuals was completed with 25 being resistant.

LAYPERSON SUMMARY
Although Pierce's disease (PD) has existed in California since the late 1800s, the introduction of the glassy-winged sharpshooter to California in the late 1990's significantly increased the spread and damage caused by PD. A collaborative breeding program was started in 2000 to develop PD resistant table and raisin grapes with high fruit quality comparable to that existing in markets today. The first crosses to make the BC4 generation of table and raisin grapes with *V. arizonica* source of PD resistance were made this year. These families will have high fruit quality as they consist of 97% *V. vinifera*. An example of increased fruit quality would be this year's selection of five raisin grapes made from BC3 *V. arizonica* families which will be propagated for production trials. An additional fifteen BC2 *V. arizonica* raisin selections were made and will be propagated for production trials. The use of molecular markers has allowed the selection of PD resistant seedlings while they are still in test tubes. Three hundred twenty-seven resistant seedlings were selected from 885 seedlings this year, thereby making the program more efficient. Advanced selections are screened in the greenhouse to verify PD resistance. Powdery mildew (PM) resistance is being combined with PD resistance and this year 54 of 97 PD resistant seedlings showed PM resistance after greenhouse screening. A family from BD5-117 source of resistance that is different from *V. arizonica* has been made to develop molecular markers for this source of resistance. To date, 154 seedlings have been tested with 70 fluorescent labeled SSR markers. The PD resistance of 125 of these seedlings has been tested in the greenhouse. This collaborative research between USDA/ARS, Parlier and University of California, Davis has the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry where PD might restrict the use of conventional table and raisin grape cultivars.

INTRODUCTION
Pierce's disease (PD) has existed in California since the late 1800s when it caused an epidemic in Anaheim. A number of vectors for PD already exist in California , and they account for the spread and occurrence of the disease. The introduction of the glassy-winged sharpshooter to California in the 1990's significantly increased the spread and damage caused by PD. Other vectors exist outside California and are always a threat. All of California's commercially grown table and raisin grape cultivars are susceptible to PD. An effective way to combat PD and its vectors is to develop PD resistant cultivars so that PD epidemics or new vectors can be easily dealt with. PD resistance exists in a number of *Vitis* species and in *Muscadinia*. PD resistance has been introgressed into grape cultivars in the southeastern United States, but fruit quality is inferior to *V. vinifera* table and raisin grape cultivars grown in California. Greenhouse screening techniques have been improved to expedite the selection of resistant individuals (Krivanek et al. 2005, Krivanek and Walker 2005). Molecular markers have also been identified that make selection of PD resistant individuals from *V. arizonica* in these families even quicker (Krivanek et al. 2006). The USDA, ARS grape breeding program at Parlier, CA has developed elite table and raisin grape cultivars and germplasm with high fruit quality. Embryo rescue procedures for culturing seedless grapes are being used to help introgress the seedless trait with PD resistance quickly (Emershad et al. 1989). This collaborative research gives the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry.

OBJECTIVES

1. Develop PD resistant table and raisin grape germplasm/cultivars with fruit quality equivalent to standards of present day cultivars.
2. Develop molecular markers for Xf/PD resistance in a family (SEUS) other than those from *V. arizonica.*

RESULTS AND DISCUSSION

Objective 1

Fifty-one crosses using *V. arizonica* and SEUS (BD5-117 and Zehnder) sources of resistance were made in 2009 and produced 5,918 berries, 6,661 ovules, and 1,719 embryos (25% embryos/ovules) (**Table 1**). The first BC4 crosses (97% *Vitis vinifera*) was made this year and consisted of 31 crosses of which 19 produced 3,931 berries, 5,000 ovules and 1,372 embryos. These crosses were made before observing fruit on the 18 month old seedlings and the size of the seed/aborted seed was unknown at bloom. The seed traces were too small to culture from six of these crosses. The seedlings obtained from these crosses should have high fruit quality as they now have 97% *V. vinifera* in their background. In additional to the BC4 crosses, 17 BC3 crosses were made and consisted of 1,313 berries, 987 ovules and 312 embryos. Five and three crosses combined *V. arizonica* and SEUS PD resistance respectively with powdery mildew resistance. No seeded crosses were made in 2009.

Leaves were taken from seedlings in test tubes in November from 2008 crosses and tested for resistance with molecular markers for the PdR1 locus on chromosome 14. Results for three BC1 and ten BC3 seedless x seedless families (89-0908 *V. arizonica* source of resistance) is shown in **Table 2**. A total of 885 individuals were tested with SSR markers and 812 showed markers on both sides of the PdR1 region as expected. A total of 327 individuals (43% of those showing markers) were resistant and planted to soil in cups for growth in the greenhouse before planting to the field in April, 2009. Thirty-nine percent of the plants showing markers were susceptible. This is very similar to the percent resistant and susceptible plants obtained for over 1,600 F1, BC1, BC2 table and raisin seedlings reported by Riaz et al. 2009. The susceptible and recombinant individuals were discarded making more efficient use of greenhouse and field space. A total of 172 seeded by seedless BC3 seedlings were also screened with molecular markers and 34% and 44% were resistant and susceptible respectively. Only the resistant plants were planted in the field. Four BC3 crosses made in 2008 combined PD resistance from *V. arizonica* with powdery mildew (PM) resistance from *V. romanetii*. Usually resistance from this source of PM resistance segregates in a 1:1 resistant:susceptible ratio. The seedlings that had PD resistant markers were screened in the greenhouse for PM resistance. Of the 97 PD resistant seedlings screened to date, 54 were resistant, which is as expected. Inoculation of plants with *Xylella* in the Greenhouse (method by Krivanek et al. 2005, Krivanek and Walker 2005) was done to determine resistance of 105 selected individuals from BC2 *V. arizonica* and F1, BC1 SEUS (Table 3). Sixty-three resistant individuals were from *V. arizonica* and only one was from SEUS source of resistance. This shows that a high level of resistance is being passed on by *V. arizonica*. Greenhouse testing is absolutely necessary to make the final decision about resistance of individual selections. The highest level of resistance is being obtained from *V. arizonica* and BD5-117 and their use as parents will be emphasized.

Fifty percent of the 400 resistant BC2 and BC3 *V. arizonica* seedlings planted in 2008 produced fruit. From these seedlings, 3 BC2 and 3 BC3 tray dried raisin, and 2 natural DOV raisin selections were made that are good enough for propagation into advanced production trials. An additional 15 tray dried raisin, 4 natural DOV raisin, 10 table grape and 2 wine selections were kept for use as parents and for additional evaluations. Raisin samples were dried from 41 PD resistant seedlings from 2 BC2 raisin families. Nine seedlings were selected for propagation in production trials and as parents. One selection had a rating of 52 which was higher than Selma Pete (50), Fiesta (48) and DOVine (47) and equal to Diamond Muscat (52). Nine table grape selections from BC2 *V. arizonica* have been selected for further observation and as parents. Four have been tested in the greenhouse for *Xylella* infection and were resistant. Five table grapes and nine raisin grapes were also selected from 227 BC2 *V. arizonica* seedlings that were planted in 2007 and fruited for the first time in 2009. Three of the raisin selections will be propagated for yield trials. Twelve advanced selections, with a range of PD resistant in greenhouse tests, have been planted in a replicated plot at the USDA ARS research station, Weslaco, Texas. Samples were taken in September, 2009 to determine if any plants have become infected after one year's growth

Objective 2

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 and 300 individuals are fruiting. Fruit samples were taken from all seedlings for cluster weight, berry weight and seed/seed trace weight as an indication of fruit quality. Greenhouse testing for PD resistance is complete on 125 individuals, with 25 being resistant (**Table 3**). Three hundred additional plants were planted this year to increase the family size to over 500 individuals. Of the 105 SSR polymorphic primers identified last year, 70 have been labeled with fluorescent dyes and run on all 154 individuals plus the parents. The additional 35 labeled polymorphic primers are being tested on the 154 seedlings.

Table 1. Successful 2009 table and raisin grape PD resistant seedless crosses and the number of ovules and embryos produced.

Female	Male	Type	No. Emas-culations	No. berries Opened	No. Ovules	No. Embryos
89-0908 *V. rupestris* x *V. arizonica*						
B71-60	07-5054-12	Table BC4	2,823	229	408	234
Scarlet Royal	07-5054-12	Table BC4	3,115	11	20	6
07-5061-04	A63-85	Raisin BC4	2 bags[a]	252	285	68
07-5061-04	A50-33	Raisin BC4	3 bags[a]	391	411	148
07-5061-14	Y143-39	Raisin BC4	3 bags[a]	256	460	69
07-5061-14	B82-43	Raisin BC4	4 bags[a]	643	1,168	97
07-5061-14	Y144-157	Raisin BC4	2 bags[a]	298	441	239
A49-82	07-5061-34	Raisin BC4	3,000	74	50	24
A50-39	07-5061-34	Raisin BC4	3,315	402	394	100
A50-91	07-5051-28	Raisin BC4	1,500	108	117	21
A50-91	07-5061-146	Raisin BC4	1,830	160	178	44
A61-79	07-5058-16	Raisin BC4	2,800	142	144	37
B82-43	07-5052-43	Raisin BC4	2,606	223	250	64
Y142-76	07-5061-34	Raisin BC4	496	42	45	14
Y142-76	07-5061-72	Raisin BC4	1,492	88	84	13
Y142-76	07-5061-106	Raisin BC4	1,156	38	31	7
Y143-161	07-5053-33	Raisin BC4	3,946	207	229	28
Y144-132	07-5061-34	Raisin BC4	2,390	32	50	10
05-5501-27	Y129-176	Table BC3	5 bags[a]	36	3	1
05-5501-27	05-5501-28	Table BC3	4 bags[a]	170	16	2
05-5501-28	Y133-191	Table BC3	1,564	21	23	4
05-5502-25	Y129-176	Table BC3	2,631	163	162	30
05-5501-68	C57-60	Table BC3	5 bags[a]	240	287	99
05-5501-68	C45-64	Table BC3	5 bags[a]	48	13	2
05-5501-40	Y129-161	Table BC3	5 bags[a]	51	52	7
05-5502-15	04-5514-2443	Table BC3	2,708	382	290	39
05-5551-19	A63-58	Raisin BC3	650	77	94	18
04-5514-28	C61-123	Table BC2	1,375	75	93	23
07-5061-14	Y308-344	PM Raisin BC4	3 bags[a]	210	235	149
Total			**39,397**	**44,256**	**6,033**	**1,597(26%)**
SEUS source of resistance (BD5-117 or Zehnder)						
03-5003-10	C45-64	Table BC1	5 bags[a]	2	2	1
03-5003-10	Y308-314	PM Table BC1	1,554	69	88	20
Y305-58	C61-123	PM Table BC1	3,097	308	436	101
Total			**5,285**	**3,291**	**4,836**	**1,179(23%)**

[a]Parents with female flowers were not emasculated, only bagged and pollinated.

Table 2. Determination of seedling resistance based on molecular markers for 89-0908 BC2 families made in 2008.

Family	Type Cross	No. Resistant[a]	No. Susceptible[b]	No. Recombinant[c]	No data[d]	Off Types	Total
08-5001	Table BC3	46	17	13	1	19	96
08-5002	Table BC1	40	35	29	9	14	127
08-5003	Table BC3	6	6	2	1	0	15
08-5054	Raisin BC1	38	37	13	5	0	93
08-5055	Raisin BC1	29	33	18	8	1	89
08-5056	Raisin BC3	27	45	14	16	0	102
08-5057	Raisin BC3	29	28	6	2	2	67
08-5058	Raisin BC3	6	2	0	3	4	15
08-6002	Table BC3 PM	35	28	18	9	3	93
08-6003	Table BC3 PM	2	2	0	2	0	6
08-6052	Raisin BC3 PM	5	3	1	6	2	17
08-6053	Raisin BC3 PM	62	59	18	11	10	160
08-6054	Raisin BC3 PM	2	2	1	0	0	5
Total		**327 (43%[e])**	**297 (39% [e])**	**133 (18% [e])**	**73**	**55**	**885**
	Seeded x Sdlss						
08-5504	Table BC3	7	8	3	4	0	22
08-5505	Table BC3	1	0	1	0	0	2
08-5552	Raisin BC3	47	66	16	6	0	135
08-6501	Table BC3 PM	3	2	5	2	0	12
08-6502	Table BC3 PM	1	0	0	0	0	1
Total		**59 (37% [e])**	**76 (48% [e])**	**25 (16% [e])**	**12**	**0**	**172**

[a]Resistant = marker on both sides of *PdR1* region.
[b]Susceptible = no *PdR1* markers.
[c]Recombinant= genotypes that amplified with one *PdR1* marker.
[d]No data = genotypes that failed to amplify properly.
[e]%= Number of seedlings in each category / total number of seedlings showing markers properly.

Table 3. Results of greenhouse test for determination of PD reaction.

Population	Resistance Source	Total sent	Testing Compete	
			No. tested	No. resistant
BD5-117 map	BD5-117	154	125	25
Arizonica	PdR1	113	105	63
Other PD	SEUS	65	45	1
Total		**332**	**275**	**109**

CONCLUSIONS

Families for the development of PD resistant seedless table and raisin grape cultivars continue to be produced. Emphasis was placed on *V. arizonica* BC3 crosses (93% *V. vinifera*) and BC1 crosses of BD5-117. The use of molecular markers has simplified and sped up the identification of PD resistant individuals from *V. arizonica.* Seedless table and raisin grape selections with PD resistance and improved fruit quality have been made in both BC2 *V. arizonica* and F1 BD5-117 families. One hundred five polymorphic SSR primers have been identified in the BD5-117 family in the search for molecular markers from sources of resistance other than *V. arizonica*. SSR primers are now being tested on all 154 individuals from the BD5-117 family to develop a frame work map. The development of PD resistant table and raisin grape cultivars will make it possible to keep these grape industries viable in PD infested areas. Molecular markers will greatly aid the selection of PD resistant individuals from SEUS populations.

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BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

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ABSTRACT
The use of marker-assisted selection (MAS) using DNA markers tightly linked with Pierce's disease (PD) resistance (see our companion report) and the acceleration of the seed-to-seed breeding cycle to three years have allowed very rapid progress towards the creation of PD resistant winegrapes. Seedlings from the 2008 crosses were screened for PD resistance with MAS and only those seedlings with the markers were planted in the field. The goals of the 2009 crosses were to: 1) create 97% *V. vinifera* seedlings with PD resistance using *PdR1* from *V. arizonica* hybrid– F8909-08; 2) create 75% *V. vinifera* seedlings with PD resistance from *V. arizonica* b40-14; and 3) enlarge the *V. arizonica/girdiana* b42-26 PD resistance mapping population by remaking the *V. vinifera* F2-35 x b42-26 cross. Numerous greenhouse-based PD resistance screens were performed on breeding lines, mapping populations and new PD resistant rootstocks. Selections with *PdR1* at the 87.5% and 75% *vinifera* level at our Beringer, Napa County trial were inoculated and a similar trail at the 93.75% *vinifera* level was planted. Finally, small-scale wine lots were made from three 93.75% *vinifera* and five 87.5% *vinifera PdR1* selections. Fruit evaluation and juice analysis were performed on numerous other promising progeny at the 93.75% *vinifera PdR1* level.

LAYPERSON SUMMARY
Rapid progress breeding Pierce's disease (PD) resistant winegrapes continues to be made by combining the use of MAS with *PdR1* and aggressive vine training to produce clusters in a seedling's second season to produce the next generation crosses of PD resistant populations. Wines were made this Fall from PD resistant selections that contain 94% *vinifera*. These selections resulted from the original cross of *vinifera* x F8909-08 (the *V. arizonica/candicans* resistance source from Monterrey, Mexico), followed by crossing back to a *vinifera* parent over three more generations (modified Back Cross 3 – mBC3). This Fall, wine was made from three of these 94% *vinifera*, PD resistant selections. Two years of wines have also been made from the previous generation 88% *vinifera* (mBC2) PD resistant selections with very favorable results. This year also saw the creation of the next generation back cross to *vinifera* (mBC4), which will result in 97% *vinifera* seedlings with *PdR1* resistance for planting in Spring 2010. PD resistant selections are also being tested at the Beringer ranch in Napa Valley, and wines will be made from these vines. Major advances have also been made in the production and greenhouse testing of seedling populations that will allow the characterization of PD resistance genes from multiple backgrounds (see companion project on the genetics of resistance to PD).

INTRODUCTION
The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xylella fastidiosa* (*Xf*) resistance (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005), and having unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, to allow the introduction of extremely high levels of *Xf* resistance into commercial grapes. They have made wine from vines that are 93.75% *V. vinifera*, and possess resistance from the b43-17 *V. arizonica*/*candicans* resistance source. There are two sources of *PdR1*, 8909-08 and 8909-17 – sibling progeny of b43-17. These selections have been introgressed into a wide range of winegrape backgrounds over multiple generations, and resistance from southeastern United States (SEUS) species is being advanced in other lines. However, the resistance in these later lines is complex and markers have not yet been developed to expedite breeding.

OBJECTIVES
1. Breed Pierce's disease (PD) resistant winegrapes through backcross techniques using high quality *V. vinifera* winegrape cultivars and *Xf* resistant selections and sources characterized from our previous efforts.
2. Continue the characterization of *Xf* resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations.

RESULTS AND DISCUSSION
Objective 1 – The breeding cycle for the development of PD resistant grapes has been reduced to three years (seed-to-seed) using MAS with the b43-17 resistance sources and their progeny. The breeding goal at this point is to introgress PD and *PdR1* resistance sources into a large number of *V. vinifera* winegrape backgrounds. Now that the backcross four (BC4) (96.9% *V. vinifera*) level has been produced (seedlings will be planted in 2010) larger numbers of progeny within populations will be produced to increase chances of selecting the best winegrape quality in a PD resistant background. **Table 1** shows the crosses made in 2009. The goals of the 2009 crosses were to: 1) use the *PdR1* allele from F8909-08 to advance the *vinifera*

winegrape populations to the 96.9% *vinifera* level; 2) create populations of 93.75% *vinifera* with the *PdR1* F8909-08 resistance allele; 3) select *V. vinifera* x *V. arizonica* b40-14 progeny and produce 75% *vinifera* populations with an alternative PD resistance source; and 4) increase the *V. arizonica/girdiana* b42-26 mapping population by remaking the *V. vinifera* F2-35 x b42-26 cross.

During this period, eight groups of plants were tested in the greenhouse for *Xf* resistance (**Table 2**). Group A tests confirmed the resistance of the parents used in the 2008 crosses, previously selected on the basis of their DNA markers; initiated the exploration of the *PdR1* alleles among resistant genotypes and *vinifera* parents; and tested the progeny from one initially promising 87.5% *vinifera* VR (*vinifera* x *rotundifolia*) hybrid from Olmo's breeding program. Unfortunately, all of these VR progeny were ELISA tested as PD susceptible at over 1,000,000 cfu/ml (data not shown), confirming the complex nature of PD resistance derived from *rotundifolia*. Groups B, D and E evaluated the greenhouse-based PD resistance of the 50% *vinifera*, 25% b40-14 *V. arizonica* resistance source 07744 and 07386 populations. **Table 3** shows that for the 07744 population, 24% were classed as resistant, approximately 26% were in either of two intermediate classes and 50% were in the susceptible class. The clearly PD resistant genotypes identified in groups B & E allowed us to make 75% *vinifera* crosses in 2009 and advance this promising new PD resistance line by another generation (**Table 1c**). Group D was tested to evaluate the impact of between pot spacing on mean ELISA cfu/ml values in an 87.5% *vinifera PdR1* background. Consistent with Baumgartel (2009), tighter spacing increased the mean ELISA values relative to the standard spacing in both susceptible and resistant selections. Group F consisted of additional 9621 population recombinants that were tested to aid the fine scale mapping of *PdR1*. Concurrently, additional 2007 crosses were tested to continue the exploration of *PdR1* resistance initiated in Group A. Groups G & H focus on the F8909-17 allele of *PdR1* to elaborate differences in resistance behavior of this allele compared to the F8909-08 allele. The new PD resistant rootstocks were tested in Group G. Chardonnay was used as a susceptible scion to determine if high *Xf* levels in the scion increase *Xf* levels in the rootstock downward across the graft union. A81-17, a rigorously tested 75% *vinifera PdR1* genotype, was used as the resistant scion to determine whether grafting on *PdR1* rootstocks impacts the titer of *X. fastidiosa* found in the scion.

Objective 2 - Although resistance from other backgrounds is complex and quantitative, which results in few resistant progeny from crosses to *vinifera* cultivars, we continue to advance a number of lines. In order to better understand the limits of other PD resistance sources the following resistance sources are being studied:

V. arizonica/girdiana b42-26 – *Xf* resistance in the 0023 (D8909-15 (*V. rupestris* x b42-26) x *V. vinifera* B90-116) population is strong, but is quantitatively inherited. Quantitative trait locus (QTL) analysis has identified a major QTL that accounts for about 20% of the variability (preliminary results). Previous efforts with this population focused on table grape breeding, and found that the 0023 population (F1, 1/4 b42-26) had about 30% resistant progeny. The 0023 population has a large number of weak genotypes, few females with viable seeds, and generally lacks fertility. The progeny of a cross of a resistant 0023 genotype crossed back to *vinifera* (BC1) were tested and only 7% were resistant. In 2007, we tested the 05347 (*vinifera* F2-35 x b42-26) population to examine the b42-26 resistance source in a background without the confounding effect of *V. rupestris*. That same year, crosses using elite *V. vinifera* wine type pollen were made to a number of females in this population and 140 genotypes were planted in 2008, which flowered for the first time in Spring 2009. We planted an additional 100 05347 genotypes to the field in May 2009 and again repeated this cross, producing approximately 200 seeds, to further expand this F1 mapping population.

V. shuttleworthii Haines City – Based on encouraging greenhouse screen results for this resistance source, in 2008 the BC1 (75% *vinifera*), and BC2 (87.5% *vinifera*) using a BC1 from earlier table grape work were made. This BC1 is very resistant and has reasonable winegrape characteristics. If reliable genetic markers for *Xf* resistance from this resistance source can be developed, it will be relatively easy to incorporate this form of resistance with sources carrying *PdR1* at later stages of the program, with the goal of broadening resistance.

V. arizonica b40-14 – Over the last seven years, 45 F1 progeny of PD susceptible *V. rupestris* Wichita Refuge crossed with PD resistant *V. arizonica* b40-14 (the R89 series) have been tested. Forty-two were highly resistant and three had intermediate reactions (data not shown). In 2006, the 06339 population (*V. vinifera* F2-35 x b40-14) was made and contains 198 seedlings for testing. In 2007, *V. vinifera* cv. Airen was crossed onto two of the PD resistant R89 series genotypes and a total of 163 progeny were planted in Spring 2008. One of these is the 07744 population (F1 50% *vinifera*, 25% b40-14 – see **Tables 1c, 2 and 3**). Preliminary mapping of this population places PD resistance from b40-14 on LG14 but in a different location than *PdR1*. To date greenhouse testing has been completed for seven 06339 genotypes (F1 50% *vinifera*, 25% b40-14); they all lack PD symptoms and have low ELISA values. The progeny of the 06339 x *V. vinifera* crosses made in 2008 will be used for further mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance.

Given that low levels of *Xf* exist in resistant plants it will be important to have PD resistant rootstocks to graft with resistant scions, thus preventing failure if *Xf* moved into the rootstock. The rooting and grafting ability (with two scion varieties) of eight selections with PD resistance from *PdR1* have been tested, and they will soon be greenhouse tested for resistance and

examination of *Xf* movement across the graft union. The best selections will be tested for nematode and phylloxera resistance followed by field testing.

Field and Wine Evaluations – Field testing of advanced PD resistant selections continues at the Beringer vineyard in Yountville, CA. Natural sharpshooter vectoring is not depended on, rather each plant is needle inoculated with *Xf* each Spring. Selections from the BC3, 94% *vinifera* crosses from the 07355 (U0505-01 x Petite Sirah) and 07370 (*vinifera* F2-35 x U0502-38) populations were grafted onto Dog Ridge (currently the only certified virus-free PD resistant rootstock) in February 2009 and planted at Beringer in June 2009. These genotypes have been marker tested and their PD resistance status will be confirmed by greenhouse testing in the coming months. This Spring, selections from the 045554 (BC2, 87.5% *vinifera*) population were needle inoculated for the second time and selections from the A81 population (BC1, 75% *vinifera*) both with the *PdR1b* (F8909-08) allele were inoculated for the third time. This year the seven most promising 87.5% *vinifera PdR1* wine types (06325-42, 06325-43, U0502-01, U0502-10, U0502-35, U0502-38, U0502-41 – 2 white and 5 red; 6 reps each) grafted onto Dog Ridge and planted at the Beringer site for small-scale winemaking trials.

In 2006, at least six vines of eight 87.5% *vinifera PdR1* selections (50% Syrah or Chardonnay from the last cross) were planted for small-scale wine making tests. Wine lots of these selections made in 2007 and 2008 have shown significant promise. Sensory evaluation as well as fruit evaluation and must analysis from numerous other genotypes from crosses involving elite wine cultivars were reported in our last two progress reports. These wines were also evaluated at the UCD Viticulture and Enology alumni gathering on May 15, 2009 and at the North American Grape Breeders Conference in Tallahassee, Florida on August 7, 2009 with similar results. In 2008, at least six vines of four other particularly promising 87.5% *vinifera PdR1* selections, siblings of the 2006 plantings, were planted. Concurrently we planted at least six vines of eight 93.75% *vinifera PdR1* selections (50% Petite Syrah, Chardonnay or F2-35 from the last cross). This fall, 12 fermentations were made: three (two red, one white) at the 94% *vinifera* level; five (four red, one white) at the 87.5% *vinifera* level; and four (two red, two white) *vinifera* and PD controls. Vine, fruit and juice analyses are presented in **Tables 4a-c** and images of the vines, leaves and fruit are in **Figures 1 and 2**. Numerous other genotypes from crosses involving elite *vinifera* wine cultivars were examined for fruit evaluation and must analysis. ETS Laboratories (www.etslabs.com) of St. Helena kindly donated their fruit analysis and phenolics panel, which uses a wine-like extraction to model a larger fermentation.

CONCLUSIONS

This project continues to breed PD resistant winegrapes with the primary focus on the *PdR1* resistance source so that progress can be expedited with MAS. Populations with *Xf* resistance from other sources are being maintained and expanded, but progress is slower with these sources. We continue to supply plant material, conduct greenhouse screens and develop new mapping populations for our companion project on fine-scale mapping of PD resistance to allow the characterization of the *PdR1* resistance locus. The first testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2007, then again in 2008 and they scored remarkably well. Small-scale wine lots from advanced selections with 93.75% *vinifera* were made in Fall 2009 and appear even more promising.

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Table 1. 2009 Wine and mapping crosses with estimated number of seeds produced.

Resistant Type	*Vinifera* Parent of Resistant Type	*Vinifera* Parents used in 2009 Crosses	Est. No. Seeds
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 96.875% *V. vinifera* parentage.			
07354-50	Merlot	Cab. Sauv., Chard.	125
07355-020	Petite Syrah	Cab. Sauv., Chard., Chenin blanc, Zinfandel	1750
07370-039	F2-35 (Cab. Sauv. x Carignane)	Cab. Sauv., Chenin blanc, Riesling, Sylvaner, Zinfandel	1450
07370-097	F2-35	Cab. Sauv., Chard., Chenin blanc, Pinot noir	650
07370-28	F2-35	Cab. Sauv., Chenin blanc, Pinot noir, Zinfandel	950
07371-19	F2-35	Cab. Sauv., Chard., Chenin blanc, Sylvaner	375
07371-20	F2-35	Cab. Sauv., Chenin blanc, Pinot noir, Sylvaner	925
07371-36	F2-35	Cab. Sauv., Chard., Chenin blanc, Mourvedre, Riesling, Zinfandel	800
1b. *Vitis arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.			
07307-10	Zinfandel	Cab. Sauv., Chenin blanc	115
1c. Crosses to the b40-14 *V. arizonica* resistance source to produce progeny that are 75% vinifera and 12.5% the resistance source.			
07744-038,-120	Airen	Cab. Sauv., F2-35, Malaga Rosada	790
1d. Cross to increase the b42-26 *V. arizonica* x *vinifera* mapping population.			
b42-26	F2-35 (Cab. Sauv. x Carig.)	F2-35	200

Table 2. PD resistant winegrape progeny completed or currently in greenhouse screening for PD resistance.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Date	Resistance Source(s)
A	2008 *PdR1* Parents, mini-mapping, e6-23 VR series	47	11/25/2008	2/26/2009	b43-17 (both alleles), VR
B	07744 mapping population	37	12/16/2008	4/2/2009	b40-14
C	Greenhouse spacing trial	NR	1/15/2009	5/21/2009	F8909-08
D	07386 Mapping population	45	2/3/2009	5/21/2009	b40-14
E	07744 mapping populations	70	2/12/2009	5/21/2009	b40-14
F	9621 recombinants, 2007 crosses of interest	122	4/21/2009	8/13/2009	F8909-08
G	04191 mapping population, PD Rootstocks	82	6/23/2009	9/22/2009	F8909-08
H	04191 mapping pop (MPP)	130	10/8/2009	1/7/2010	F8909-08

Table 3. Greenhouse screen results for the 07744 cross (R8918-05 x Airen).

Resistance Class	Resistance Class Parameters	No. in Class	% Total
R	mean cfu/ml ~<100k	26	24%
R?	mean cfu/ml >~100k and max cfu/ml <1M cfu/ml	9	8%
S?	mean cfu/ml >~300k and <~3M cfu/ml	18	17%
S	mean cfu/ml >~1M and max cfu/ml >3.5M	54	50%
Total		107	100%

Table 4a. Phenotypic observations of reference varieties and select progeny with the *PdR1* resistance source used for small lot winemaking in 2009.

Genotype	Parentage	Percent *vinifera*	2009 Bloom Date	Berry Color	Berry Size (g)	Ave Cluster Wt. (g)	Ripening Season	Prod 1=v low, 9=v high
Barbara	Historic	100%	05/09/09	B	2.4	290	late	6
Chardonnay	Gouais blanc x Pinot noir	Historic	05/14/09	W	1.0	190	early	5
07355-12	U0505-01 x Petite Sirah	93.75%	05/10/09	B	1.0	137	early-mid	6
07355-75	U0505-01 x Petite Sirah	93.75%	05/07/09	B	1.3	234	early	8
07713-51	F2-35 x U0502-48	93.75%	05/07/09	W	1.4	210	early	8
U0501-12	A81-138 x Syrah	87.50%	05/18/09	B	1.1	194	late	4
U0502-10	A81-138 x Chardonnay	87.50%	05/07/09	B	1.4	198	early	7
U0502-20	A81-138 x Chardonnay	87.50%	05/14/09	W	1.7	313	late	8
U0502-26	A81-138 x Chardonnay	87.50%	05/10/09	B	1.6	375	mid	7
U0505-35	A81-138 x Cab. Sauvignon	87.50%	05/10/09	B	1.1	158	early	6
Blanc du Bois	Fla D6-148 x Cardinal	~66%	05/14/09	W	1.2	125	mid-late	7
Lenoir	*V. aestivalis* hybrid	<50%	05/20/09	B	0.8	201	late	6

Table 4b. Analytical evaluation of advanced selections with the *PdR1* resistance source used for small lot winemaking in 2009. All analysis courtesy of ETS Laboratories, St. Helena, CA.

Genotype	L-malic acid (g/L)	°Brix	potassium (mg/L)	pH	TA (g/100mL)	YAN (mg/L, as N)	catechin (mg/L)	tannin (mg/L)	Total antho-cyanins (mg/L)
07355-12	2.79	26.8	2050	3.42	0.78	275	127	585	2178
07355-75	2.88	28.2	2180	3.49	0.74	217	5	680	1941
07713-51	1.31	23.4	1700	3.56	0.49	146	-	-	-
U0501-12	2.11	21.8	1610	3.46	0.58	263	49	555	1026
U0502-10	3.97	24.9	2170	3.60	0.73	362	48	1006	1162
U0502-20	4.18	23.3	2230	3.51	0.76	383	-	-	-
U0502-26	2.24	24.0	1900	3.40	0.73	237	67	411	947
U0505-35	4.03	28.7	2450	3.66	0.81	476	47	886	1446

Table 4c. Sensory evaluation of reference varieties and advanced selections with the *PdR1* resistance source used for small scale winemaking in 2009.

Genotype	Juice Hue	Juice Intensity	Juice Flavor	Skin Flavor	Skin Tannin (1=low, 4= high)	Seed Color (1=gr, 4= br)	Seed Flavor	Seed Tannin (1=high, 4= low)
Barbara	pink-brown	low	neutral, acidic	jam, berry	2	4	nutty, spicy	3
Chardonnay	green-gold	medium	apple, pear	sl fruity	1	4	nutty	4
07355-12	red	med-dark	red fruit	plum, berry	3	3.5	woody, spicy	1
07355-75	red	medium	plum, fig	jam,prune	2	3	hot, woody	2
07713-51	green-gold	medium	apple, pear	neutral	2	3.5	woody, spicy	3
U0501-12	red	med-dark	fruity	fruit jam	2	4	neutral	2
U0502-10	pk-red-orng	med-dark	slight vegetal	sl fruity	1	4	nutty, spicy	1
U0502-20	green	medium	neutral, fruity	grass	1	4	spicy, bitter	1
U0502-26	pink	medium	bright, spicy	fruity	2	4	nutty	3
U0505-35	red	medium	CS-veg, berry	sl CS-veg	2	4	spicy	2
Blanc du Bois	gold	med-dark	floral, vegetal	sl vegetal	1	4	spicy, bitter	4
Lenoir	red	dark	mildly fruity	fruity	1	4	nutty	4

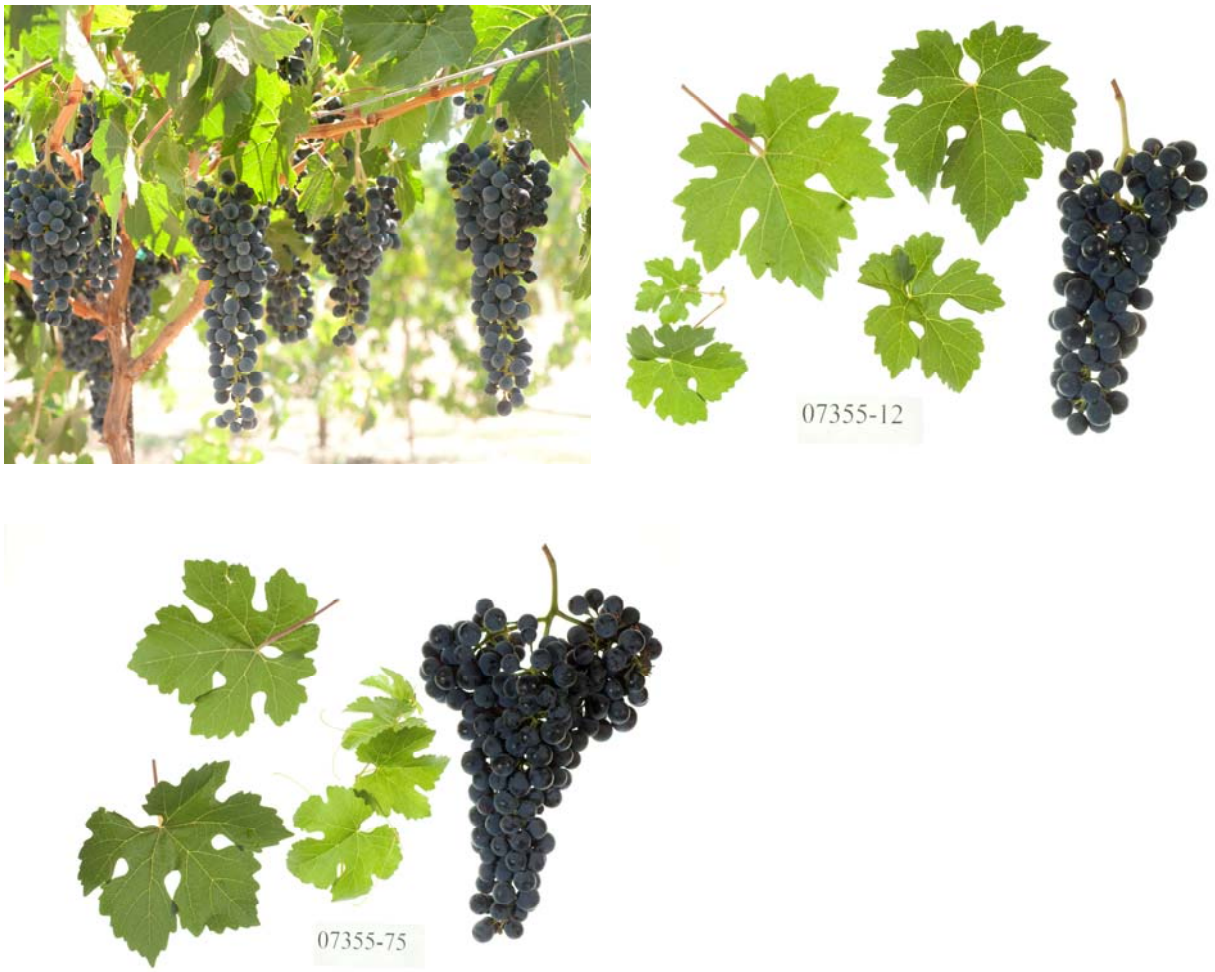

Figure 1. Images of the two red 94% *vinifera* PD resistant winegrape selections (U0505-01 x Petite Sirah) used for small-scale winemaking at UCD in Fall 2009. 07355-12 is above and 07355-75 is below.

Figure 2. Images of the white 94% *vinifera* PD resistant wine grape selection (F2-35 (Cabernet Sauvignon x Carignane) x U0502-48) used for small-scale winemaking at UCD in Fall 2009.

MAP-BASED IDENTIFICATION AND POSITIONAL CLONING OF *XYLELLA FASTIDIOSA* RESISTANCE GENES FROM KNOWN SOURCES OF PIERCE'S DISEASE RESISTANCE IN GRAPES

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Reporting Period: The results reported here are from work conducted August 2009 to October 2009

ABSTRACT
This report presents updated results on the refined mapping of the Pierce's disease (PD) resistance locus, *PdR1*, in the 04190 (397 plants) and 9621 population (900 plants). In both populations, the resistance locus is within a 1cM distance. The flanking markers VVCh14-78 and VVCh14-81 were added to key recombinant plants from both populations and greenhouse screening was repeated to avoid any error. Genetic mapping was initiated in the 07744 (resistance from *V. arizonica* b40-14), 04191 (resistance from F8909-17 *PdR1a*) and 05347 (resistance from *V. arizonica/girdiana* b42-26) populations. A total of 152 markers were completed for 07744 to develop the framework map and greenhouse screening of the 07744 population is complete and underway for the 04191 population. In 07744, preliminary analysis indicated that PD resistance (*PdR1c*) resides on chromosome 14, in the same region where *PdR1a* (resistance from F8909-17) and *PdR1b* (resistance from F8909-08) mapped from the b43-17 background. However, the SSR alleles for resistance are very different between b43-17 and b40-14. Between October 2007 and March 2009 two BAC libraries, each with one restriction enzyme (*Hind* III and *Mbo* I), were completed and the screening of the *Hind* III BAC library with flanking markers was initiated. The Pinot noir genome sequence was used to develop markers to screen the BAC library, and these SSR markers were used to reduce the physical distance to *PdR1*. Two screenings of the libraries identified 24 (with markers VVCh14-56 and VVCh14-10) and 17 positive BAC clones (with marker VVCh14-58). Complete sequencing of two clones (H23P13 and H64M16), representing the two haplotypes of b43-17 was completed. Five clones were positive with VVCh14-56 and VVCh14-58. Clone 'H69J14' (which is bigger than 200Kb) was selected for 454-based sequencing. This clone spans scaffold 21 and nine of the Pinot noir genome sequence. A total of 42,000 sequences were assembled with the help of two different assembly programs. The DNASTAR program was used to obtain assembly at 99% stringency, and yielded more than 79 contigs larger than 5Kb in size. Primers will be designed from the ends to improve the assembly with BAC walking by filling the gaps and verifying the sequences on the ends of contigs. Assembled sequence will be used for the identification of resistance gene(s).

LAYPERSON SUMMARY
Genetic mapping efforts have identified a Pierce's disease (PD) resistance region on chromosome 14 termed *PdR1*, which originated from *Vitis arizonica/candicans* b43-17. This resistance acts as a single dominant gene and we have mapped the two forms from the homozygous parent – *PdR1a* from F8909-17 and *PdR1b* from F8909-08. We have also mapped another form of *PdR1* from *V. arizonica* b40-14, and are examining how the multi-gene PD resistance from *V. arizonica/girdiana* b42-26 maps and relates to *PdR1*. In the future these multiple resistance forms will be combined in our PD breeding program to ensure the strongest resistance possible. The combination of these forms of PD resistance can only be done with the tightly linked genetic markers discovered in these mapping efforts so that the combination of the various forms of resistance can be confirmed in the interbred progeny. These mapping efforts are also essential to physically locating and characterizing PD resistance genes. At present, the chromosome region that *PdR1* exists on has been sequenced and these pieces of sequence are being arranged and compared to the Pinot noir genome sequence and that of other plants to characterize their function and determine which are likely to be involved in PD resistance.

INTRODUCTION
This project continues to genetically map Pierce's disease (PD) resistance in forms of *V. arizonica* (Riaz et al. 2007). These efforts are closely linked to a breeding program focused on developing PD resistant winegrapes (see companion report). The breeding program produces and greenhouse screens the seedling populations upon which this genetic mapping program depends. While the tightly linked genetic markers generated in these mapping efforts are used to optimize and greatly accelerate the PD breeding program (Riaz et al. 2009). These markers are also essential to the successful introgression of resistance from multiple sources, and thus for the production of broader and more durably resistant grapevines (Riaz et al. 2008a). Genetic maps associate DNA markers with phenotypic traits, and allow the linking of these traits with markers positioned relative to each other on linkage groups, which since the sequencing of the Pinot noir genome, are now known to be chromosomes. Fine scale mapping of given regions and careful screening of recombinant progeny (those with a given genetic marker but without resistance, or vice versa, because of a recombination event) is critical to the identification of relatively short genetic regions that can then be sequenced so the genes responsible for PD resistance can be characterized and their function studied (Riaz et al. 2008b).

OBJECTIVES

1. Completely characterize and refine the PD resistance locus on chromosome 14 by genetically mapping in four populations that derive resistance from *V. arizonica/candicans* b43-17 and its *V. rupestris* x b43-17 progeny F89090-08 (*PdR1b*) and F8909-17 (*PdR1a*): 04190 (*V. vinifera* F2-7 x F8909-08), 9621 (D8909-15 x F8909-17), 04191 (F2-7 x F8909-17), and 04373 (*V. vinifera* F2-35 x *V. arizonica/candicans* b43-17).
2. Genetically map PD resistance from other forms of *V. arizonica*: b42-26 (*V. arizonica/girdiana*) and b40-14 (*V. arizonica*).
3. Develop a BAC (bacterial artificial chromosome) library for the homozygous resistant genotype b43-17 (parent of F8909-08, and F8909-17) and screen the library with closely linked markers.
4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1a* gene candidates.

RESULTS AND DISCUSSION

Objective 1. We previously reported that the genetic position of the *PdR1a* resistance locus was between marker VVCh14-56 and VVCh14-70. In the past three months, we have developed three additional SSR markers derived from Pinot noir genome sequence that allowed us to narrow down the physical distance from 300Kb to 200 Kb. These markers (VVCh14-77, VVCh14-78 and VVCh14-81) were added to the composite set of recombinants from the 9621 population as well as to the resistant and susceptible parents used for crosses in 2008 to determine if the resistance allele is unique and not present in susceptible selections. The resistance allele was unique in size, which made these markers very valuable and robust for marker-assisted screening. There are three key recombinants from the tested set of more than 900 plants. For two plants, the recombination event happened between VVCh14-78 and *PdR1a*, and other plant had a recombination event between VVCh14-81 and *PdR1a*. With the addition of new markers, the *PdR1a* locus is within a 1cM window and it completely correlates to the physical distance between the markers that were developed from Pinot noir genome sequence.

F8909-08 possesses the *PdR1b* resistance locus, which is being mapped in the 04190 population. Previously we reported that *PdR1b* maps between VvCh14-02 and VVCh14-70. Additional markers (VvCh14-28/VVCh14-29/VVCh14-30) were added to the entire set of 397 plants in the 04190 population. The greenhouse screen was repeated for key recombinants, which also helped refine the data. In addition, marker analysis identified 14 recombinants from 15 different crosses (1,000 plants) based on resistance from F8909-08. We completed the greenhouse screen on 35 recombinants (including seedlings from *PdR1b* background crosses). The screen identified four key recombinants: in two plants the recombination event occurred between *PdR1b* and VVCh14-02; and in one plant the recombination event occurred between *PdR1b* and VVCh14-70. The greenhouse screen is being repeated for four other recombinants that had inconclusive first test results. In the most updated map, we have placed the *PdR1b* locus between markers VVCh14-81 and VVCh14-78 (**Table 1**). Both of these markers are less than 200Kb apart from each other based on the Pinot noir genome sequence.

The 04191 population (*V. vinifera* F2-35 x F8909-17) has 153 progeny and a population where resistance from F8909-17 (*PdR1a*) can be examined without possible confounding effects from D8909-15 (since D8909-15 has a multigenic resistance from b42-26). The resistance locus *PdR1a* is mapped in the 9621 (D8909-15 x F8909-17) population, and the 04190 population mentioned above, and refined mapping focused only on chromosome 14. The 04191 population will be critical for the identification of any minor genes that might contribute to resistance. Therefore, we are expanding the framework genetic mapping to all 19 chromosomes, and the 153 plants in 04191 will be greenhouse screened. Greenhouse results will be available in Spring 2010 and the framework map will follow.

Objective 2. Previous mapping and greenhouse screen data from the 0023 population (D8909-15 x *V. vinifera*) with resistance from *V. arizonica/girdiana* b42-26 found that PD resistance is quantitative. The 05347 population (b42-26 x *V. vinifera*) was created to better study this resistance source. A total of 337 markers were tested on a small parental data set. Results found a high level of homozygosity for b42-26 (only 113 markers were polymorphic); 184 markers were homozygous for the male parent b42-26, 40 markers did not amplify. A total of 70 markers were added on a set of 64 progeny, and many will have marker data soon. The current population size is 165 and crosses were made to increase the population size this Spring. Greenhouse screening results will be available in Summer 2010, and a framework map will be developed on a core set of 165 seedlings; this population is being expanded for the future mapping of this quantitative PD resistance trait.

Vitis arizonica b40-14 is a third promising resistance source with PD resistance that seems to be homozygous and controlled by a single dominant gene. Previously, we reported that all F1 progeny from a cross of *V. rupestris* x b40-14 (the R8918 population) were resistant except three genotypes with intermediate results. Two resistant siblings were used to develop two populations: 07388 (R8918-02 x *V. vinifera*) and 07744 (R8918-05 x *V. vinifera*). We completed DNA extractions from 122 seedlings of the 07744 and 105 seedlings from the 07386. A total of 277 markers were polymorphic for one or the other parent in preliminary marker screening. One hundred fifty two polymorphic markers were completed on the entire set of 122 plants in the 07744 population. Mapping analysis was carried out on each parent separately. The framework map of R8918-05 was produced with 152 markers on 121 genotypes with JOINMAP (3.0). Only three markers were unlinked and the remaining 149 markers were grouped into the expected 19 chromosomes. QTL analysis was performed with MAP QTL (4.0)

and the Kruskal-Wallis approach was used to complete the preliminary analysis. No association with PD resistance was found on any other chromosome except 14 – the same chromosome where *PdR1a* and *PdR1b* map. PD resistance from b40-14 (which we have named *PdR1c*) also maps in the same general region between flanking markers VVCh14-78 and VVIN64 and within 1.5 cM. The LOD threshold for the presence of this QTL was very high (**Table 2**). Next, interval and MQM analysis will be carried out after the selection of markers as cofactors, to determine the level of variance contributed by this b40-14 based resistance locus.

Objective 3 and 4. Two BAC libraries (each from different restriction enzymes) created from the homozygous resistant b43-17 were developed. In the first phase of the project, library screening was carried out with markers (VVCh14-10 and VVCh14-56), both tightly linked to *PdR1*. This process identified 24 positive clones – four of the clones were positive with both markers: H23-P13, H34-B5 and H64-M16 and H45-J22. New markers (both SSR and non-repetitive) were developed from the 695Kb region from the Pinot noir genome sequence covered by markers VVCh14-56 and VVCh14-70/77/78 (see previous reports). This region overlaps two different scaffolds from the Pinot noir genome sequence (9 and 21). Currently, *PdR1* is placed between Ch14-81 and Ch14-78 at a physical distance of ~200Kb. Based on the genetic map from the 9621 population, the physical and genetic distance correlates because 1cM is equivalent to about 216Kb. The second round of BAC library screening was carried out with the Ch14-58 marker. A total of 17 clones were positive, five of them were also positive with the VVCh14-56 marker (see details in previous report). Clone H69J14 was selected for 454 sequencing. A total of 42,000 sequences were generated. Two different programs were used to assemble the sequence. The DNASTAR program seqman allowed assembly at a stringency level of 99%, which generated more than 4,000 contigs representing 38,000 sequences. A total of 79 sequences were bigger than 5 KB. **Table 3** presents the contigs that are bigger than 6 Kb and have sequence similarity to the Pinot noir genome sequence (**Figure 1**). It is important to note that the sequence similarity to scaffold 21 of Pinot noir was almost 98% identical for most of the contigs, however, the b43-17 sequences that overlap with scaffold nine of Pinot noir matched to multiple sites and level of similarity was less. This result suggests that either the b43-17 genomic region with the PD resistance gene(s) is divergent from Pinot noir, or the 8X assembly of Pinot noir's scaffold 9 has lots of gaps and errors. The 12X coverage of Pinot noir genome would be more helpful to conduct meaningful sequence comparison. In the next step, primers will be designed to fill the gaps, improve the coverage and to verify the sequences at the ends of the contigs.

CONCLUSIONS

Genetic mapping efforts have identified valuable genetic markers for marker-assisted selection and enabled rapid progress towards PD resistant winegrapes (see companion report). These mapping efforts have now identified three alleles of *PdR1*: *PdR1a* and *PdR1b* derived from *V. arizonica/candi*cans b43-17; and *PdR1c* derived from *V. arizonica* b40-14. These alleles were found to map within the same general region, but suggest that although PdR1 seems to be a single gene trait, the region may be composed of a number of tightly linked genes. BAC library sequence analysis of b43-7 is resulting in candidate genes suggestions for *PdR1* and these are being compared to the Pinot noir genome sequence and to similar regions in other plants. The genomic characterization of this region will help us determine how this form of PD resistance functions and which genes control it.

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Table 1. Key recombinants from the 9621 (*PdR1a*) and 04190 (*PdR1b*) populations. The genotypes in bold red font are key recombinants with a recombination event between the marker and the resistance locus. "0" indicates a susceptible allele and "1" indicates a resistant allele.

Genotypes with *PdR1a* background	A010	VVCh 14-56	VVCh1 4-81	*PdR1a*	VVCh 14-78	VVCh 14-77	VVCh 14-70	VVCh14 -29	VMCNg2 b7.2
-416	0	0	0	0	0	0	0	1	1
-426	0	0	0	0	0	0	0	1	1
-470	0	0	0	0	0	0	0	1	1
-554	0	0	0	0	0	0	1	1	1
-1064	1	1	1	1	1	1	0	0	0
-8	0	0	0	**0**	**1**	1	1	1	1
-194	0	0	0	**0**	**1**	1	1	1	1
-38	0	0	**0**	**1**	1	1	1	1	1
-15	1	1	1	1	1	1	1	0	0
-23	1	1	1	1	1	1	1	0	0
-901	1	1	1	1	1	1	1	0	0
-915	1	1	1	1	1	1	1	0	0
-919	0	0	0	0	0	0	0	1	1

Table 1. Cont'd.

Genotypes with *PdR1b* background	VVCh 14-10	VVCh 14-02	VVCh 14-81	PdR1 b	VVCh 14-78	VVCh 14-77	VVCh 14-70	VVCh 14-30	VVCh 14-27
06314-24	0	0	0	0	0	0	0	1	1
06328-05	0	0	0	0	0	0	0	1	1
04190-026	0	0	0	0	0	0	0	1	1
06317-50	1	1	1	1	1	1	1	0	0
04190-383	1	1	1	1	1	1	1	0	0
06317-50	1	1	1	1	1	1	1	0	0
04190-320	1	1	1	?	1	1	1	0	0
04190-065	1	1	1	?	1	1	1	0	0
04190-109	1	1	1	1	1	**1**	**0**	0	0
04190-381	1	1	1	**1**	**0**	0	0	0	0
06711A-60	0	0	0	?	1	1	1	1	1
04190-236	1	1	1	?	0	0	0	0	0
06315-49	1	0	0	0	0	0	0	0	0
06326-23	1	0	0	0	0	0	0	-	-

Table 2. The Kruskal-Wallis analysis LOD values for the PdR1c locus in the 07744 population based on resistance from *V. arizonica* b40-14.

Genetic map	Map locus	K* (df)
0	VVIN70	5.392 (1) **
3.5	VVIn94	9.323 (1) ****
9.5	ctg1025882	16.293 (1) *******
10.4	VVIP26	12.764 (1) ******
10.7	VVIS70	17.315 (1) *******
11.6	UDV025	16.160 (1) *******
15.0	VVIN64	21.081 (1) *******
16.7	VVCh14-78	22.692 (1) *******
16.7	VVCh14-77	22.946 (1) *******
17.7	VVCh14-70	19.350 (1) *******
20.3	VMCNg2b7.2	17.282 (1) *******
21.5	VVMD24	20.496 (1) *******
22.0	VMC5b3	20.631 (1) *******
22.5	VMC2a5	22.915 (1) *******
22.5	VVIV69	21.978 (1) *******
23.2	UDV033	22.857 (1) *******
28.9	VMC6c10	15.577 (1) *******
36.2	VMC2c3	8.872 (1) ****
36.5	VMC2b11	8.057 (1) ****
36.9	VChr14a	7.229 (1) ***
39.0	ctg1008359	8.772 (1) ****
39.8	VMC9f4	9.360 (1) ****
41.1	VMC2h12	8.967 (1) ****
49.8	VMC1e12	3.507 (1) *
59.2	VVIP05	1.714 (1) -
61.1	VChr14b	0.398 (1) -
65.4	VVC62	0.386 (1) -

Table 3. Contig size, level of similarity and match location to Pinot noir contigs 9 and 21. Majority of the contigs that were similar to scaffold 9 matched to different locations. The similarity of the b43-17 sequence is greater when it is closer to scaffold 21.

Contig number	Contig length (Kb)	Number of sequences	Coverage	% Match to Pinot noir	Scaffold	Start position
4154	11,020	158	5.74	98	21	15,201,490
2454	11,011	108	3.55	94	9	15,002,217
2620	10,109	87	3.01	98	21	15,165,403
2801	9,741	82	3.36	97	21	15,183,600
1824	9,375	96	3.55	93	9	15,096,377
1834	9,324	113	4.49	98	21	15,200,996
2554	8,240	78	3.60	92	9	multiple sites,
440	8,177	64	3.14	95	9	15,103,036
673	8,066	73	3.09	98	21	15,175,438
2410	7,779	84	4.04	96	9	15,078,745
3944	7,703	69	3.57	98	21	15,165,453
3654	7,463	61	3.05	98	21	15,180,883
2411	7,288	84	4.36	97	21	15,208,989
3773	7,269	157	8.19	98	21	15,179,580
2341	7,267	102	5.89	-	-	-
1658	7,247	49	2.63	-	-	-
1918	7,230	62	3.14	90	9	multiple sites,
3997	7,226	141	7.2	92	9	multiple sites,
45	7,217	66	3.59	87	9	multiple sites,
1734	6,996	170	9.68	98	21	15,180,952
1885	6,985	87	4.77	-	-	-
496	6,945	90	5.09	99	21	15,181,699
530	6,763	319	19.75	90	9	multiple sites,
3606	6,713	57	3.17	98	21	15,181,366
959	6,647	87	4.8	99	21	15,168,301
420	6,599	99	6.07	92	9	multiple sites,
1259	6,593	155	9.28	-	-	-
2510	6,585	66	3.76	-	-	-
4108	6,545	66	3.91	98	21	15,168,284
1741	6,463	43	2.24	91	9	15,002,306
1216	6,410	184	12.05	94	9	15,070,922
3562	6,354	103	6.42	90	9	15,004,525
2610	6,331	83	4.94	97	21	15,168,523

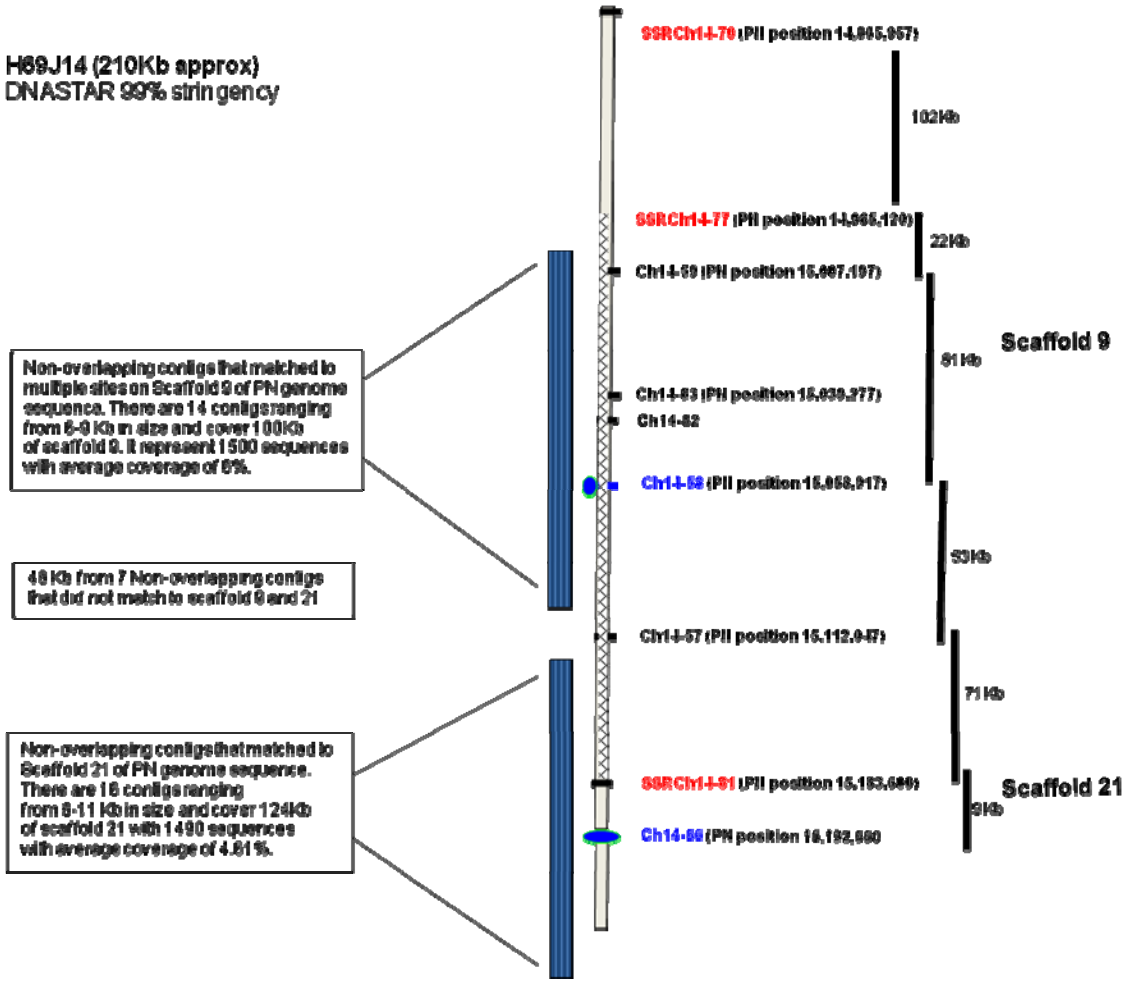

Figure 1. Assembly detail of the H69J14 BAC clone that spans two scaffolds from the Pinot noir genome (scaffold 21 and 9). Currently the PD resistance locus resides between SSR markers Ch14-81 (on scaffold 21) and Ch14-77 (on scaffold 9). The relative position and distance of all the markers that have been used in mapping and library screening are on the right. Non-overlapping contigs were grouped based on their position on the Pinot noir. Primers will be developed for the contig ends to enable BAC walking in order to fill the gaps and verify the sequences.